THE FLORIDA STATE UNIVERSITY

COLLEGE OF ARTS AND SCIENCES

FLUORESCENT STAINING OF BIOTINYLATED ?-DNA ATTACHED TO STREPTAVIDIN-COATED PARAMAGNETIC PARTICLES

By

NORA A. PEPPER

A Thesis submitted to the
Department of Chemistry
in partial fulfillment of the requirements for Honors in the Major

Degree Awarded:
Fall Semester, 2005

The members of the Committee approve the thesis of Nora A. Pepper defended on April 14th, 2005

_____
Dr. Thomas Fischer
Professor Directing Thesis

_____
Dr. Cynthia Lundeen
Outside Committee Member

_____
Dr. Oliver Steinbock
Committee Member

ABSTRACT

This experiment is designed to prepare ?-DNA for experimentation by attaching it to a paramagnetic particle. The protocol in Appendix 2 was formulated to provide an inexpensive and relatively simple method of preparing a fluorescently-labeled, biotinylated DNA sample that could then be attached to streptavidin-coated paramagnetic particles. The goal is to prepare a complex in which each paramagnetic bead is permanently attached to a single DNA molecule, and each of these bound DNA molecules has a fluorescent tag, enabling the visualization of the molecules via fluorescence microscopy. Once formed, this complex will be able to be easily manipulated in a variety of media. Optical tweezers or magnetic trapping may be used to stabilize the beads, fixing the beads while the DNA is freely controllable for study.

If this method is successful, the experiment may hold some significance for the scientific community. Primarily, it may facilitate the isolation and study of individual DNA molecules. By allowing single DNA molecules to be stretched and measured, it may provide a simple and economical method for manipulating individual DNA molecules. This could have an impact on research areas involving quantifying DNA, such as genome mapping, or exploration of DNA, such as gene therapy.

The experiment has four distinct stages: 1) ?-DNA is labeled with biotin, which binds to the 3' end of the DNA, enabling the DNA to bind to the streptavidin surface of the paramagnetic particle; 2) the excess biotin in the sample that did not bind with the DNA is removed; 3) ?-DNA is stained with one of three fluorescent dyes: PicoGreen, YOYO-1 or YOYO-3; and 4) the biotinylated, fluorescent DNA strands are attached to paramagnetic particles that have been coated with streptavidin.

Once the ?-DNA has been attached to paramagnetic particles, it will be possible to observe and analyze the DNA by exploiting the magnetic properties of the beads to manipulate the DNA-bead complex. An example of this would be to use a strong magnet to stabilize the beads in a fluid flow, and adjusting the flow to observe the stretching of the individual molecules.

INTRODUCTION

This experiment is designed to prepare ?-DNA for study by attaching it to a paramagnetic particle. The experimental procedure outlined in Appendix 2 was formulated to provide an inexpensive and relatively simple method of preparing a fluorescently-labeled, biotinylated DNA sample that could then be attached to streptavidin-coated paramagnetic particles. The goal is to prepare a complex in which each paramagnetic bead is permanently attached to a single DNA molecule, and each of these bound DNA molecules has a fluorescent tag, enabling the visualization of the molecules via fluorescence microscopy. Once formed, this complex will be able to be easily manipulated in a variety of media. Optical tweezers or magnetic trapping may be used to stabilize the beads, fixing the beads while the DNA is freely controllable for study.

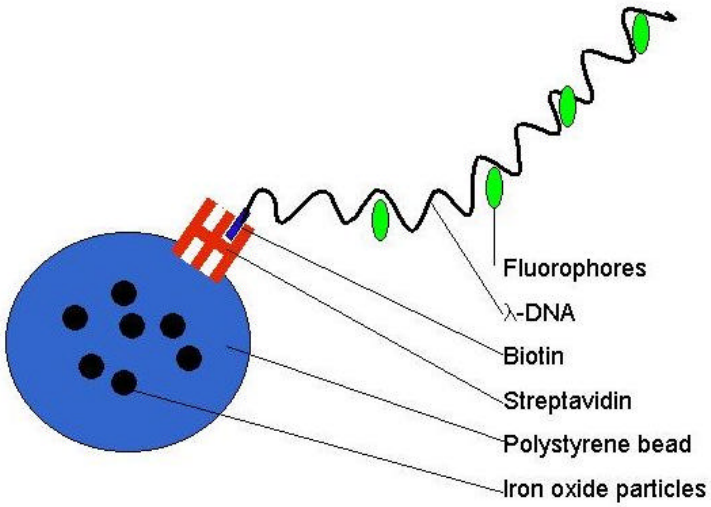

Figure 1. Schematic representation of the experiment.

If the method is successful, this experiment may hold some significance for the scientific community. Primarily, it may facilitate the isolation and study of individual DNA molecules. By allowing single DNA molecules to be isolated and stabilized, it may provide a simple and economical method for manipulating individual DNA molecules for analysis. This could have an impact on research areas involving quantifying DNA, such as genome mapping; or on exploration of DNA, such as gene therapy.

The experiment has four distinct stages, yielding a dye-DNA-bead complex. A schematic drawing of this system is shown in Figure 1. In the first step, ?-DNA is labeled with biotin, which binds to the 3' end of the DNA, thereby enabling the DNA to bind to the streptavidin surface of the paramagnetic particle.

In the second step, the excess biotin in the sample that did not bind with the DNA is removed. Should free (unbound) biotin remain in the sample, it would bind to the streptavidin-coated beads at essentially the same rate as the bound biotin, thereby reducing the binding sites available for the biotinylated DNA. Having beads in sample with only biotin attached would greatly hinder the ability locate beads with DNA attached to them. Therefore, removal of the unbound biotin is an important step in ensuring a pure sample of the DNA-bead complex.

In the third step, ?-DNA is stained with one of three fluorescent dyes: PicoGreen, YOYO-1 or YOYO-3. The stains permanently bind to the ?-DNA, thus making the ?-DNA visible when viewed with a fluorescence microscope. These three dyes were chosen because their emission/excitation spectra were well-suited for the laboratory

conditions available. They are but a few of the myriad fluorescent dyes commercially available.

In the fourth step, the biotinylated, fluorescently-labeled DNA strands are attached to paramagnetic particles that have been coated with streptavidin. The streptavidin has a high affinity for biotin, creating a permanent, stable bond between the DNA and the paramagnetic particle.

Once the ?-DNA has been attached to paramagnetic particles, it will be possible to observe and analyze the DNA by exploiting the magnetic properties of the beads to manipulate the DNA-bead complex. For instance, a strong magnet may be used to stabilize the beads in a fluid flow, and the flow rate adjusting to observe the effect on the individual DNA molecules.

OVERVIEW OF COMPONENTS

DNA

The nucleic acid chosen for this experiment is ?-phage DNA. The DNA was chosen for its large size and its prevalence. Because it is widely used in experimentation, its properties are well-documented. ?-DNA is a bacteriophage nucleic acid comprised of 48,502 basepairs (48.5 kbp), meaning that the individual DNA molecules are very large, approximately 16 μm, when fully stretched. Using a large DNA molecule, as opposed to a smaller one, increases the likelihood of visualization, thus facilitating the observation and measurement of individual DNA molecules and increasing the overall success of the experiment.

Due to the extensive nature of the experiment, it was broken into stages, the first of which was to visualize the DNA alone. To do this, two techniques were incorporated. The first involved visualizing concentrated DNA that had been air-dried and placed on a glass slide, while the second used a Brewster Angle Microscope to view concentrated DNA on the air/water interface. The results of these experiments are described in this section.

Visualization of Concentrated DNA in Air

Before the addition of fluorescent stain and before the biotinylation processes, it was necessary to observe the concentrated DNA alone. To this end, a small amount of concentrated DNA (approximately 2 μL) was placed on a glass slide and was viewed with the Leica DHLP Polarization Microscope as the droplet dried. The DNA which was randomly distributed in its coiled form in the droplet stretches out and spreads toward the contact line as the liquid droplet evaporates. Fig. 2 shows the concentrated DNA after it was completely dry. The DNA appears as wavy lines radiating in virtually the same direction as the evaporative flow. The contact line is the darker region in the upper right-hand corner. These are not individual DNA strands, but instead self-assembled structures generated by the evaporative flow driving the DNA towards the contact line.

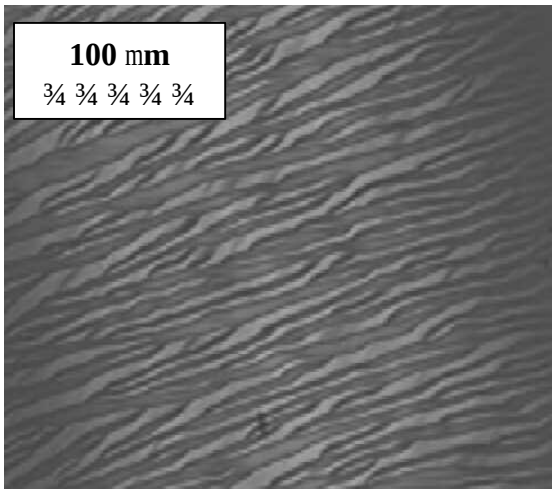

Figure 2. Concentrated DNA that has been allowed to dry on a glass slide visualized via Leica DHLP Polarization Microscope.

Visualization of Concentrated DNA on Air/Water Interface

In a similar experiment, concentrated DNA was visualized as a monomolecular film using a Brewster Angle Microscope (BAM). This was accomplished by applying a high concentration of DNA standard, (80 µg) to the subphase of a pure air/water interface via a 2-mL pipette. The DNA was allowed to stand in the trough for approximately 40 hours, after which the DNA adsorbed to the surface where it formed a structural monolayer surface. The DNA was visible as self-assembled structures of circular symmetry located at the air/water interface. Figure 3 below shows the concentrated DNA as numerous light-colored rings.

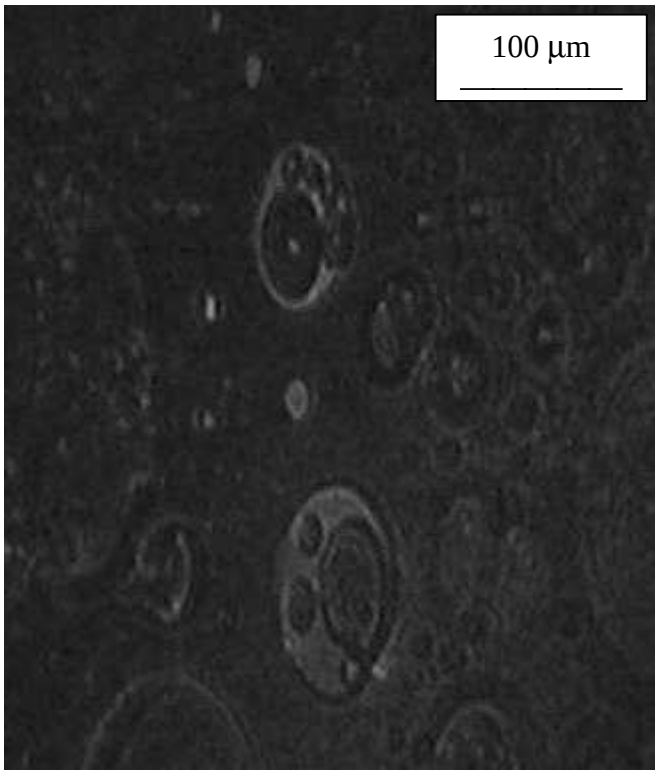

Figure 3. Brewster Angle Microscope image of a concentrated DNA monolayer.

The success of this experiment may be due in part to the long adsorption time of the DNA (approximately 2 days). This method may not be practical for use with fluorescently-stained DNA, as the protocols for the dye recommend that the stained samples be used within a day of preparation for best results.

Biotin-Streptavidin Bonding

One of the primary goals of this experiment is to find an effective method of attaching the DNA molecules to paramagnetic particles. Since there is nothing inherent about these two species that would make them compatible, a "glue" is needed to stick the two particles together. Biotin-streptavidin technology was incorporated into the experimental protocol for this purpose.

Bonding between biotin and streptavidin bond is widely used in scientific research. It was chosen for this application because the properties of biotin-streptavidin bonding are well-known and the strong bonds created should enable the DNA molecules to permanently adhere to streptavidin-coated paramagnetic particles. Biotin and streptavidin have a high bonding affinity; the attraction between these molecules creates very strong, stable bonds that can tolerate severe extremes in temperature and pH. Organic solvents and other denaturing agents also have little effect on the strong biotin-streptavidin bond, so it is safe to use in a variety of experimental conditions [1,6].

For this application, the DNA will be biotinylated; that is, biotin will be chemically attached to the 3' end of each DNA molecule [3]. The biotinylated DNA will then bond with streptavidin particles that coat the surface of each paramagnetic particle. In this way, a permanent bond will be made between the DNA and the paramagnetic particles.

Paramagnetic Particles

In order to observe and manipulate the DNA under the microscope, the molecules must be attached to something allowing them to be stabilized. To achieve this, the biotinylated DNA is attached to paramagnetic particles. These are small spheres that can be purchased as mono-dispersed collodial solutions with diameters ranging from 100 nm to 100 μm. They are made of a polymer shell encompassing iron oxide particles, which are evenly distributed inside the sphere [27]

In this procedure, the desired paramagnetic particles are those that have had their surfaces coated with a thin layer of streptavidin. The particles that will be used in this application are Dynabeads M-270 Streptavidin from Dynal Biotech, made from hydrophilic carboxylic acid containing 14% iron content. The size of the beads is 2.8 +/- 0.2 μm. The free biotin binding capabilities exceed 700 pmol/μg [26]. These beads were chosen for their uniformly spherical shape. Many other commercially available beads exist, however, their shapes are not as consistent, and many of the beads tend to have bumpy surfaces, which may have a deleterious effect on their overall performance.

Once bonded, any number of methods may be incorporated to stabilize the beads and allow the attached DNA to be manipulated and measured. One method is to use a strong magnet to hold the beads in place for study. Another method is to use optical tweezers to secure the paramagnetic particle. Optical tweezers are a type of optical trap that use a strongly focused laser to hold small particles on the order of μm [17].

FLUORESCENCE

Fluorescence Microscopy

Fluorescence microscopy utilizes the intrinsic properties of fluorescent molecules to detect intracellular structures, and is used to detect structures within a cell that would ordinarily be indistinguishable from the rest of the cell when viewed via traditional microscopy techniques. For instance, using a regular microscope, a cell nucleus would not be differentiable from the cell body when viewed with an ordinary microscope.

However, once stained with a highly specific fluorescent dye, the nucleus becomes visible in a fluorescence microscope with filters designed for the appropriate wavelength. Fluorescent molecules absorb light at a specific wavelength and emit light at a different specific wavelength. During the absorption stage, electrons of the molecule increase to a higher energy level, entering an excited state. Excited electrons are unstable and will revert to the ground state, emitting energy in the form of light as they return to normal. This phenomenon is known as fluorescence. The emitted light has less energy than the absorbed light, due to loss of some energy as heat or nonradiative transitions. Thus, the emitted light has a longer wavelength than the absorbed light. Since the absorption spectra and the emission spectra are known quantities, they can be used in fluorescence microscopy.

The Fluorescence Process

Fluorescence results from a three-stage progression that occurs in certain molecules called fluorophores. These molecules are usually polyaromatic hydrocarbons or heterocycles. A fluorophore that has been designed to concentrate in a specific region of a biological sample or to react to a specific stimulus is called a fluorescent probe. The simple electronic-state diagram of Figure 4 demonstrates the process that accounts for the fluorescence of fluorophores.

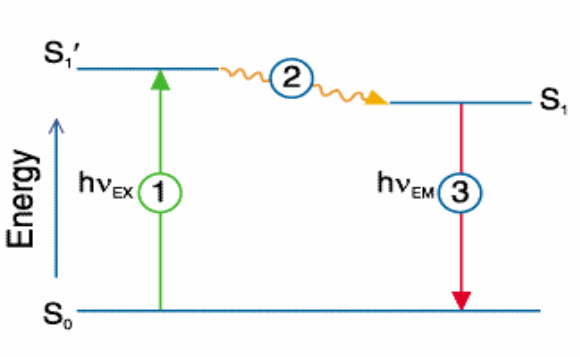

Figure 4. Jablonski diagram showing the three steps of the fluorescence process. The labels 1, 2 and 3 correspond to excitation, excited-state lifetime, and emission, respectively [16].

Step 1: Excitation

In the first step, excitation, an external source such as an incandescent lamp or a laser supplies a photon of energy $h\nu_{EX}$. The photon is absorbed by the fluorophore, which creates an excited electronic singlet state (S_1').

Step 2: Excited-State

The second stage in the fluorescence process is the excited state. It exists for approximately 1–10 nanoseconds, during which the fluorophore experiences conformational changes and is susceptible to many interactions with its molecular environment. These events have two important effects. For one, the energy of S_1' is partly dissipated, producing a relaxed singlet excited state (S_1) where fluorescence emission is derived. In addition, some of the molecules initially excited by absorption (Step 1) do not return to the ground state (S_0) by fluorescence emission. This and other factors lead to a reduction in population of the S_1 state. The ratio of the number of fluorescence photons emitted (Stage 3) to the number of photons absorbed (Stage 1) is

known as the fluorescence quantum yield, which is a measure of the relative degree to which these processes occur.

Step 3: Fluorescence Emission

The third step is fluorescent emission. In this stage, a photon of energy $h\nu_{EM}$ is emitted, which returns the fluorophore to its ground state S_0. The energy of this photon is lower due to energy dissipation that occurs in the excited-state. Because its energy is lower, its wavelength is therefore longer than that of the excitation photon $h\nu_{EX}$.

The Stokes shift, which is defined as the difference in energy or wavelength represented by ($h\nu_{EX} - h\nu_{EM}$) contributes to the sensitivity of fluorescence techniques by allowing emission photons to be detected against a low background, isolated from excitation photon [16].

Fluorescent Stain

The entire fluorescence process is dependent on the intrinsic properties of fluorophore molecules. By modifying the fluorophores, many novel nucleic acid stains have been developed which are designed to excite and emit at specific spectra and to bind expressly to parts of nucleic acids. As the processes for designing these stains are refined, developers are able to create dyes with greater and greater degrees of specificity. Today, hundreds of highly specific and extremely sensitive dyes exist, allowing researchers the freedom to choose the appropriate dye for the application.

Many cyanine dyes are readily available with varying wavelengths of emission. These wavelengths range from blue to near infrared, as shown in the figure below. The dyes may be chosen based on their excitation/emission spectra to coordinate with the microscopy techniques available.

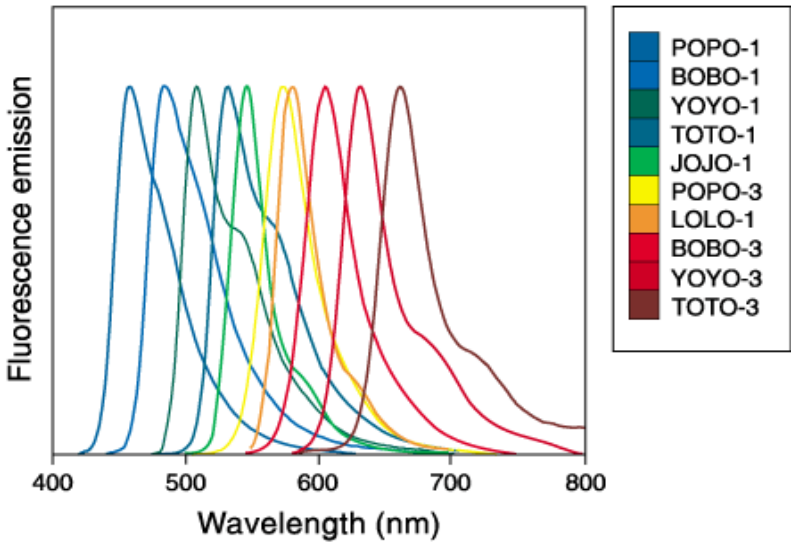

Figure 5. Normalized fluorescence emission spectra of DNA-bound dimeric cyanine dimers[16].

This experiment uses three types of sensitive nucleic acid fluorescent stains. All of the dyes in this study were provided by Molecular Probes. This company supplies an exhaustive list of nucleic acid stains, many of which are in the category of cyanine derivatives. There are four classes of proprietary cyanine dyes, including stains for ultrasensitive nucleic acid quantitation; cell-impermeant stains (the TOTO, TO-PRO and SYTOX groups of dyes); cell-permeant stains (the SYTO group); and chemically reactive stains that used to form bioconjugates (the SYBR group) [20].

There are several benefits to using cyanine dye derivatives as opposed to classic nucleic acid stains, such as ethidium bromide or propidium iodide. These benefits include: a high affinity for nucleic acid binding, a low inherent fluorescence in absence of

nucleic acids, extensive fluorescence enhancements when bound to DNA, and large molar absorptivity.

The high affinity for nucleic acids means that there is little likelihood that the stains will bind to cells other than nucleic acids. The low inherent fluorescence indicates that the stains will not fluoresce until they are bound to the nucleic acids. The quantum yield, or the ratio of light emitted from a sample to light absorbed by the sample, is generally less than 0.01 before binding to DNA. The stains show marked increases in fluorescence when they are bound to DNA, often over 1000 times stronger, with quantum yields up to 0.9. Finally, the stains have large molar absorptivity. At visible wavelengths, the extinction coefficients are usually higher than $5x10^4$ $cm^{-1}M^{-1}$ [20].

All of these properties make cyanine dyes desirable for this application. Of the many dyes available, YOYO-1, YOYO-3, and PicoGreen were chosen because their excitation/emission spectra corresponded to the laboratory conditions. YOYO-1 and YOYO-3 both belong to the TOTO series of dyes. The stains in this class are symmetric dimers of cyanine dyes having a high sensitivity for nucleic acids. PicoGreen, on the other hand, belongs to the premier class of fluorescent stains providing ultrasensitive detection of nucleic acids. The structure and molecular formulas for YOYO-1 and YOYO-3 are readily available; however, the structure and formula of PicoGreen is proprietary information and is not accessible by the general public. Therefore, much of the structure of PicoGreen must be deduced experimentally.

The cyanine dyes are all very similar in their basic structures. Minor changes, such as modifying the aromatic rings and altering the number of carbon atoms linking the

cyanine monomers, alter the dyes in important ways. These small chemical changes produce large shifts in the fluorescent spectra, while maintaining the dyes' high affinity for nucleic acids. These changes are evident when comparing structure and properties of YOYO-1 and YOYO-3.

YOYO-1 iodide (Figure 6) has one carbon atom bridging the aromatic rings of the oxacyanine dye and has an absorption/emission peak of 491/509 nm (Figure 7) when bound to double strand DNA [20].

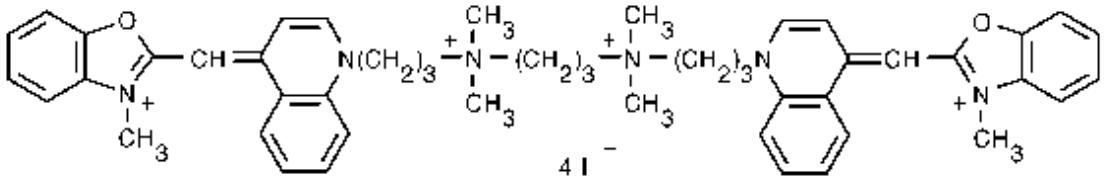

Figure 6. Structure of YOYO-1. [20]

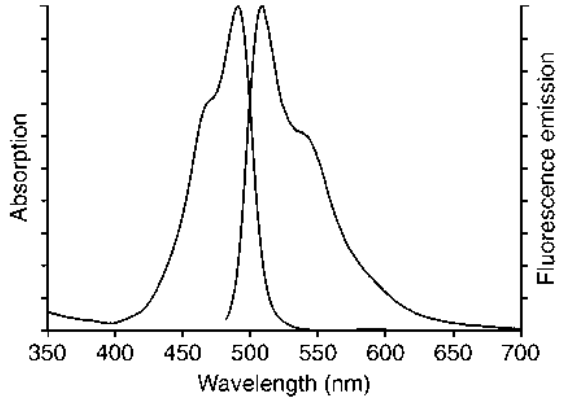

Figure 7. Spectra of YOYO-1. [20]

YOYO-3 (Figure 8) differs from YOYO-1 only by the number of carbon atoms bridging the aromatic rings; however, it exhibits an absorption/emission peak of 612/631 nm (Figure 9) under the same conditions.

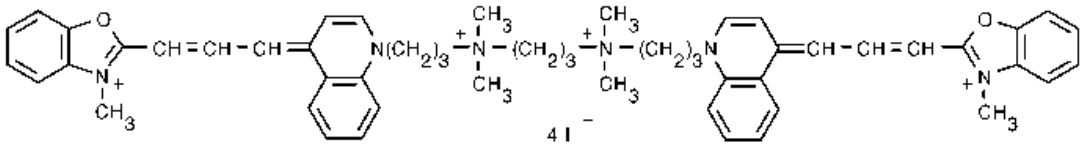

Figure 8. Structure of YOYO-3. [20]

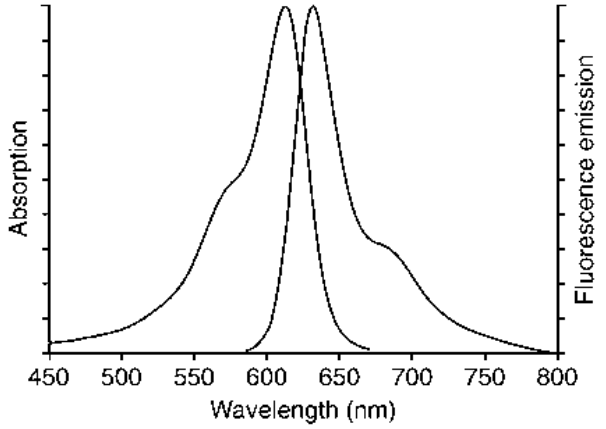

Figure 9. Spectra of YOYO-3. [20]

Based on our knowledge of the structure and spectra for the YOYO-1 and YOYO-3 dyes, YOYO-3 was chosen as the one most suitable for our laboratory conditions. YOYO-3 was thus used in a simple experiment designed to visualize concentrated, fluorescently-stained DNA.

Visualization of the DNA-YOYO-3 complex

A concentrated DNA-YOYO-3 complex was prepared by adding 40 µL YOYO-3 to 200 µL of concentrated DNA solution (500 µg/mL), yielding a basepair:staining molecule ratio of 4:1. This solution was allowed to set for approximately 45 minutes and then a small droplet (2 µL) of solution was placed on a sterile slide and viewed with a

Leica DHLP Polarization Microscope, equipped with a fluorescence filter. The DNA flowed quickly to the outer edge, or contact line of the water droplet. This method allows visualization of the DNA that is concentrated to the perimeter of the droplet; however, it is not a sufficient method to visualize an individual DNA molecule.

The following fluorescence microscope images (Fig. 10 and Fig. 11) show the results. The red curve in the photos indicates the contact edge of the droplet. The dark red area represents the stained DNA molecules, indicating that the DNA has concentrated there.

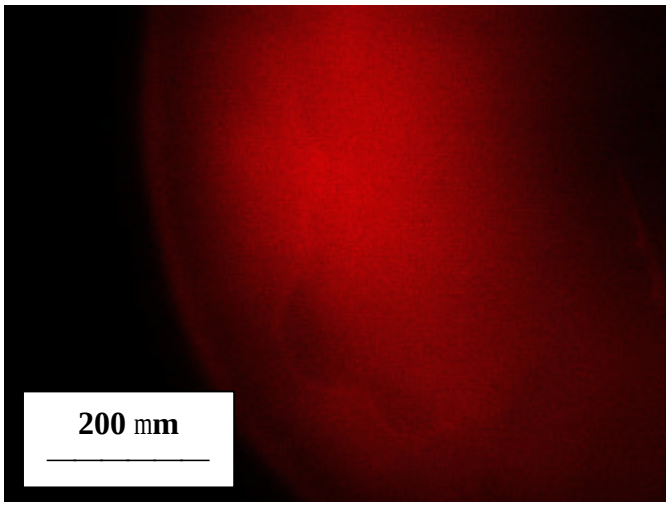

Figure 10. Fluorescence microscope image of the DNA+YOYO-3 complex, visualized via Leica DHLP Polarization Microscope equipped with proper wavelength filter, showing concentrated DNA accumulating toward the contact edge of the droplet.

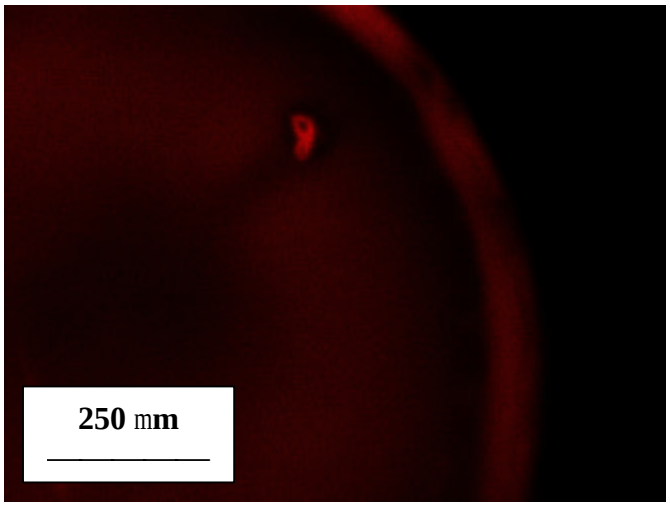

Figure 11. Fluorescence microscope image of the DNA+YOYO-3 complex, visualized via Leica DHLP Polarization Microscope equipped with proper wavelength filter, showing the DNA concentration at contact edge of droplet.

Effect of Salt Concentration on PicoGreen

The effect of salt concentration on the fluorescent properties of PicoGreen stain is of considerable interest since a variety of salty environments may occur in practical systems. However, our knowledge about these properties is limited, so we aimed to investigate how the addition of sodium chloride affects the fluorescent nature of PicoGreen. To this end, the fluorescence spectrum of a control sample was obtained, followed by the fluorescence spectra for the same sample with varying levels of sodium chloride concentrations.

Three samples were measured against the salt-free control, ranging in molarity from 0.1 to 5.0 M. The experimental protocol used is outlined in Appendix 1. As evidenced by the graphs in Figs. 12 and 13, a decrease in the peak intensity occurred as the NaCl was increased. Figure 12 shows the dampening of the fluorescence spectrum,

while the normalized graph of relative intensities versus the salt concentration is shown in Figure 13.

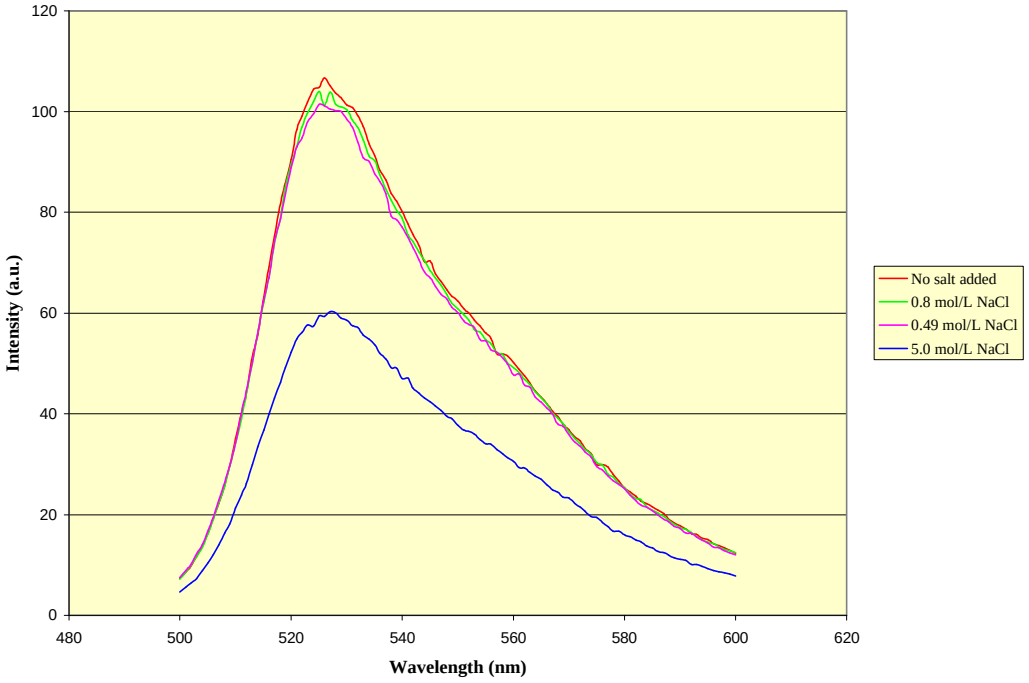

Figure 12. Fluorescence intensity versus wavelength for different concentrations of sodium chloride.

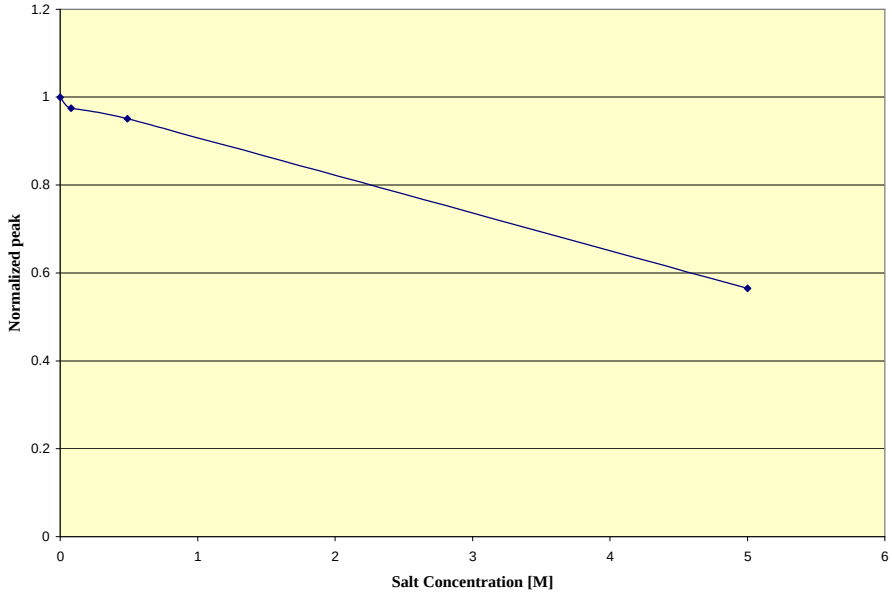

Figure 13. Normalized graph showing the relative intensities of fluorescence decrease as the concentration of NaCl is increased.

Zhu et al.[36] describe how the addition of NaCl has a dampening effect the fluorescence intensity of a hydrophobic thiacyanine dye. In this study, it was observed that minimal dampening occurred when less than 50 mM of NaCl was added. However, with the addition of higher concentrations of NaCl, further fluorescence quenching occurred. Adding higher concentrations of NaCl increases the ionic strength, and this increase causes the binding ability of the fluorescent stain to diminish. This decrease in binding ability may be due to the fact that differences in ionic strength affect the molecular conformations of DNA molecules. As ionic strength increases, the Na^+ ion protects the phosphate anion located on the backbone of the DNA molecule, which hinders the unraveling of the double strands, thus blocking the binding of fluorescent stain.

The same dampening effect that was analyzed by Zhu et al. [36] in the thiacyanine dye was observed in PicoGreen dye as well. As demonstrated by the graph in Figure 12, the highest peak occurred when the salt concentration was low; that is, before the addition of NaCl. The lowest peak coincided with the highest salt concentration. This indicates that PicoGreen dye is hindered from binding to the nucleic acid as ionic strength increases, in much the same way as the thiacyanine dye.

Although there was a dampening of the fluorescence intensity as salt concentration was increased in the PicoGreen-DNA complex, there was no change in the peak wavelength. In other fluorescent dyes, there is often a shift in spectra that occurs when the salt concentration is varied. With PicoGreen, however, there was no shift in

spectra. This may be indicative of the fact that PicoGreen stain is a non-charged compound. This is rather interesting, given the strong negative charge of the DNA.

Binding of Dyes

The binding of a fluorescent dye molecule to a nucleic acid molecule works in one of three ways: via intercalative binding, groove binding, or long range assembly on the molecular surface [36]. These binding methods may overlap to an extent depending on such factors as pH level, molecular symmetry of the dye, and ionic strength of the buffer. In intercalative binding, the dyes attach between the basepairs of the DNA molecule.

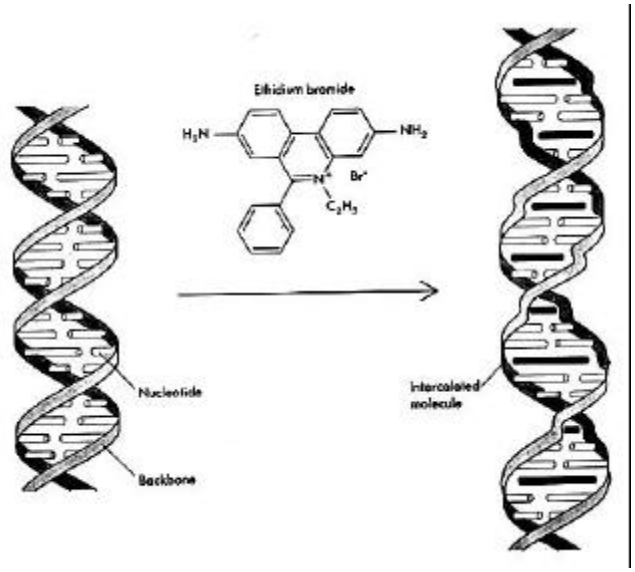

Figure 14. Intercalation in a classic nucleic acid stain. Figure courtesy: http://cmgm.stanford.edu/biochem201/Slides

Fig. 14 above shows how ethidium bromide, a classic nucleic acid stain, is intercalated between the basepairs of a DNA molecule. Analysis of YOYO-1 shows that this fluorescent stain binds in at least two ways. The predominant binding method at low dye:base pair ratios is bis-intercalation. The dye molecule monomer fits between the pyrimidines and the quinolinium ring between the purine rings. This action causes the

DNA helix to unwind [20]. At higher dye:base pair ratios a different, lesser-known binding method predominates.

Experimental Protocol

Many studies have been done in which fluorescently-labeled DNA have been visualized successfully via microscopy. In one such study, done at Stanford University, individual DNA molecules were visualized as they were stretched and relaxed in a fluid flow. Figure 15 shows a fluorescence microscope image by Thomas Perkins of Stanford University of the relaxation of a single DNA molecule stained with YOYO-1 dye, imaged at 4.5 second intervals. This experiment used optical tweezers to trap a 1 μm polystyrene sphere with a single 39 μm–long DNA molecule attached. A fluid flow was used to stretch the attached DNA to its full extension, and when the fluid flow was stopped, the stretched DNA relaxed to its original state because of its entropic elasticity

Figure 15 was created using similar fluoresence microscopy to that of our lab. The DNA-YOYO-1 complex was excited with a 488 nm argon-ion laser and visualized through a Hamamatsu SIT camera with image processing using a 515 nm longpass optical filter [22].

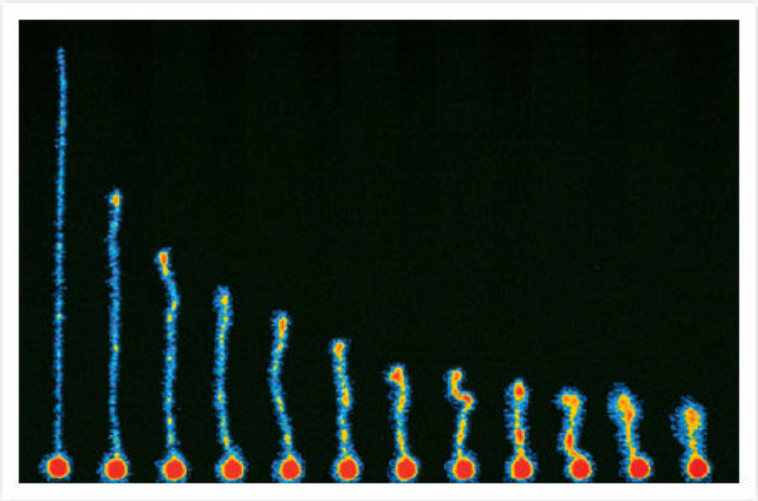

Figure 15. Stretched DNA+YOYO-1 Complex [22].

Due to the success of this and other studies, it was therefore speculated that the microscope in our lab, a Fluorescence Microscope (Zeiss Axiovert) combined with a home built Langmuir trough, would be sufficient for the visualization of the DNA-dye complex. This microscope has the appropriate sensitivity, but it differs from usual microscopes in that the conventional base has been replaced with a Langmuir trough in which to create a liquid monolayer. During experimentation, it was found that the stained DNA was not able to be stabilized in the water trough and visualized as individual molecules.

The procedure used for the biotinylation of DNA and attaching it to streptavidin-coated paramagnetic particles is outlined in Appendix 2, along with several notes indicating possible sources of error and locations for variations in the technique.

The purpose of this experiment is to prepare ?-DNA for experimentation by attaching it to a paramagnetic particle. Once the fluorescently-labeled, biotinylated ?-DNA-streptavidin bead complex is formed, it may be possible to observe and analyze the

DNA by using magnetic fields and optical tweezers to manipulate the paramagnetic particles.

The experiment was carried out as described, however visualization of the individual DNA molecules did not occur. Without a sufficient method to analyze the success of the experiment, it is not known whether this is an effective protocol. The failure to visualize single molecules is most likely due to the microscopy techniques used.

More experimentation is needed, especially in the step that involves the removal of free biotin, and an effective method of gauging success is necessary before a strong conclusion may be drawn. Nevertheless, this procedure is primarily a compilation of several credible techniques, and therefore, it is likely that this is a useful protocol for preparing samples of DNA to be observed and manipulated via strong magnets, optical tweezers, or various other trapping techniques, given the appropriate microscopy conditions.

Conclusion

The objectives of this Honors Thesis were to design an experimental protocol, and to visualize the dye-DNA-bead complex. At a solid/air interface, the λ-DNA adsorbs in minutes. This adsorbate at the solid/air interface was visualized (Figure 2). At an air/water interface, DNA adsorbs on a time scale of 2 days and forms a structural monomolecular film that was observed using Brewster Angle Microscopy (Figure 3). DNA was stained with several fluoresence dyes (YOYO-1, YOYO-3, and PicoGreen) and visualized in solution. Then DNA was bound to paramagnetic streptavidin-coated

colloidal particles using the streptavidin biotin bond. It was then stained with PicoGreen, however, visualization of a single DNA attached to a bead has not been successful. It is probable that the experiment outlined in Appendix 2 is indeed a simple and inexpensive way to prepare the dye-DNA-bead complex for study. Since we successfully visualized λ-DNA under various circumstances, I feel confident that, given more time, the visualization of a single λ-DNA could be carried out to fruition.

APPENDIX 1. Procedure for Studying the Effect of NaCl Concentration on PicoGreen.

Procedure.

1. 40 mL of DNase-free TE-buffer, 10 mM Tris ? HCl, 1 mM EDTA (pH ˜ 7.5-8.0) was prepared by diluting 0.4 mL of 100×TE buffer from SIGMA in 39.6 mL ultrapure water.

2. A single sample of DNA + PicoGreen complex was prepared by adding 400 μL of concentrated DNA to 500 μL of TE-buffer. 100 μL of PicoGreen was added. This yielded 1 mL of solution, which is a sufficient amount to fill up the lower part of the cells used in the spectrometer.

3. The control sample was placed in the spectrometer, and the fluorescence spectrum was measured.

4. To obtain a NaCl concentration of 0.1 M, it is necessary to add 2.8 mg of solid NaCl to the DNA-PicoGreen complex. The actual amount added was 2.3 mg, resulting in a NaCl concentration of 0.08 M (*2.3 mg/ 28 g mol^{-1}= 8.2x10^{-5} mol. 8.2x10^{-5} mol/1 mL = 0.0821 M).* The sample was then placed in the spectrometer and the spectrum was obtained.

5. To get a salt concentration of 0.5 M, it is necessary to add enough solid NaCl to get a total of 14 mg. In this case, 11.7 mg was needed, and the actual amount added to the sample was 11.6 mg, yielding a new NaCl concentration of 0.49 M. The fluorescent spectrum of this sample was acquired.

7. Next, to get a sample having approximately 5.0 M salt concentration, a total of 140 mg must be added to the original sample, so we needed to add 126 mg more to get this total. The actual amount added was 126.2 mg NaCl, generating a salt concentration of 5.0 M. The spectrum of this last sample was obtained.

8. The graph of these 4 salt concentrations is shown in Figure 12. The normalized graph of their relative intensities is shown in Figure 13.

APPENDIX 2. Procedure for Biotinylation of DNA, Fluorescent Staining, and Attachment to Streptavidin-Coated Paramagnetic Particles.

I. Label DNA with Biotin[13].

Materials.

1. **5´TdT Reaction Buffer**
   Included in kit
2. **Terminal Deoxynucleotidyl Transferase (TdT)**
   included in kit
3. **Biotin-N4-CTP**
   included in kit
4. **Ultrapure water**
   Have in lab.
5. **1 µM solution of oligonucleotide to be labeled**
   We will use Lambda DNA from New England BioLabs. The DNA is supplied as a standard solution of 500 µg/mL. This is equivalent to 0.016 µM.

Calculation: (500 µg/mL)(1000 mL/1 L)(1 mol/31.5 $\times 10^6$ g)(1 g/ 10^6 µg) = 1.6×10^{-8} M = 0.016 µM

The procedure calls for "5 pmol of 3'-OH ends of the sample DNA". Since each ?-DNA molecule has one 3' end, we should use 5 pmol of ?-DNA. This is approximately 315 µL of DNA standard solution (DNA is supplied as a standard solution of 500 µg/mL).

6. **37º C heat block or water bath**
   Have in lab.
7. **0.2 M EDTA, pH 8.0**
   Have solid Disodium EDTA (Ethylenediaminetetraacetic acid), 99.995% in lab; must prepare a 0.2 M solution.
8. **Chloroform:isoamyl alcohol (24:1)**
   Have in lab.
9. **Microcentrifuge Tubes**
   Have centrifuge tubes (not micro) in lab.
10. **Microcentrifuge**
    Have centrifuge (not micro) in lab.

Procedure.

IMPORTANT: TdT is a carcinogenic substance. Handle with extreme care, using the proper safety procedures. Wear a lab coat, gloves, and safety goggles while working with TdT. Thaw the 5×TdT Reaction Buffer, and Biotin-N4-CTP by placing them in refrigerator overnight. Do not take the TdT out of the freezer until just before use. It does not need to be thawed. Prepare the heat block or water bath. Use a glass beaker to hold the plastic container. Prepare 10 mL of 0.2 M solution of EDTA by adding 0.74448 g of solid Disodium EDTA to a 10 mL glass bottle. Add ultrapure water to the 10 mL mark and mix well.

Calculation: *0.2 M = 0.2 mol Disodium EDTA/1 L of solution.*

0.01 L × (0.2 mol Disodium EDTA/1.0 L solution) = 0.002 mol Disodium EDTA.

0.002 mol Disodium EDTA × 372.24 g/1 mol Disodium EDTA = 0.74448 g Disodium EDTA

Dilute the TdT in 1×TdT Reaction Buffer by combining 2 µL 5× TdT Reaction Buffer, 7 µL ultrapure water and 1 µL 20 U/µL stock TdT. This will yield 10 µL of dilute TdT solution. Prepare the labeling reaction for the sample system by adding components in the following order:

25 µL	ultrapure water
10 µL	5× TdT Reaction Buffer
315 µL	Lambda DNA standard solution (5 pmol 3' OH ends)
5 µL	5 µM Biotin-N4-CTP
5 µL	2 U/µL diluted TdT

Heat solution at 37º C for 30 minutes. May need to heat for longer period of time, because the concentration of the DNA sample we are using is much smaller than the protocol recommends **[1]**. Add 2.5 µL 0.2 M EDTA to each solution to stop the reaction. Add 50 µL chloroform:isoamyl alcohol to the solution to extract the TdT. Vortex the reaction, then centrifuge for 1-2 minutes. Remove and save the top (aqueous) phase **[2]**. At this point, the DNA is ready to be used **[3]**.

II. Remove all unbound biotin from DNA sample[29].

Any biotin that remains in the sample that did not bind with the DNA may bind to the receptor sites on the streptavidin-coated beads, thus reducing the available binding sites for the biotinylated DNA. For greater binding of the DNA, it is necessary to remove any unbound biotin.

I have found several techniques to remove the free biotin from our sample. All are different types of chromatography. The technique that I believe will be most

effective for this application is dialysis using Pierce Biotechnology's Slide-A-Lyzer MINI Dialysis Units. These kits are specifically designed for use with small sample amounts.

Materials.

1. **Slide-A-Lyzer MINI Dialysis Units**
   From Pierce Biotechnology; provided as 10K MWCO (Molecular Weight Cut-Off), 50 units.
2. **DNase-free TE-buffer**
   From SIGMA (T9285-100mL); provided as 100 ´ Concentrate, 0.2 µm filtered, 1.0M Tris ? HCl, pH ˜ 8.0 containing 01. M EDTA.

Procedure.

Place the unit into the float so that the bottom of Place the unit into the float so that the bottom of the dialysis unit is in contact with the dialysate. Always make sure that the volume level of the sample is at or above the level of the dialysate. If the volume level of the sample is lower then the level of dialysate, hydrostatic pressure will force dialysate into the unit, diluting the sample. Although the physical capacity of the unit is 500 µl, for best results, apply a sample volume of 10-100 µl with a standard pipette. To prevent contamination, do not touch the membrane with ungloved hands. If glycerol removal is desired, soak the Slide-A-Lyzer® MINI Dialysis Unit in 1 L of water for 15 minutes. Cap the Slide-A-Lyzer® MINI Dialysis Unit and place in a flotation device. Place the MINI unit's membrane in contact with dialysis buffer in a beaker, microtube or plate format. Use a low speed setting on a stir plate so that the flotation device is not submerged. Typical dialysis time to obtain equilibrium is 10 minutes to 2 hours using a dialysate volume of 0.5-1 L. For the best volume recovery, collect the sample from the corner of the Slide-A-Lyzer® MINI Dialysis Unit with a standard pipette [4].

III. Stain DNA with fluorescent dye [24,25].

Of the many commercially available fluorescent dyes, PicoGreen, YOYO-1 and YOYO-3 were chosen because they have excitation/emission wavelengths suitable for the laboratory conditions.

To Stain with PicoGreen Dye (502/523):

Materials.

1. **PicoGreen Dye**
   From Molecular Probes (P11495); provided as individual aliquots, 10 × 100 µL
2. **DNase-free TE-buffer**
   From SIGMA (T9285-100mL); provided as 100 × Concentrate, 0.2 µm filtered, 1.0M Tris ? HCl, pH ˜ 8.0 containing 01. M EDTA
3. **?-DNA**
   From New England Biolabs; provided as a standard solution of 500 µg/mL.
4. **50-mL plastic beaker**
   From Fisher Scientific.

Procedure.

Wear gloves when working with DNA. Work in the dark to prevent the dyes from bleaching. Use plastic containers for the following procedure, as the fluorescent dye may adsorb to glass. Prepare 40 mL of DNase-free TE-buffer, 10 mM Tris ? HCl, 1 mM EDTA (pH ˜ 7.5-8.0) by diluting 0.4 mL of 100 × TE buffer from SIGMA in 39.6 mL ultrapure water. Add 100 µL (one entire aliquot) of PicoGreen dye to 19.9 mL of the TE buffer previously prepared.

Use the diluted TE-buffer to prepare a 2 µg/mL solution of ?-DNA of the biotinylated DNA working solution (carried over from the previous step). The correct proportions can be calculated once the volume of biotinylated DNA solution is known. Put 1 mL of the 2 µg/mL DNA solution (from Step f) in a clean plastic container. Add 1 mL of the PicoGreen working solution to get a final DNA concentration of 1 µg/mL. Mix the solution well by gently shaking the container. Store the solution in the dark. It should ideally be used within the day of preparation. If higher concentrations of DNA or smaller volumes are needed, you may adjust the measurements above accordingly.

To Stain with YOYO-1 Dye (491/509):

Materials.

1. **YOYO-1 Dye**
   From Molecular Probes (Y3601); provided as YOYO-1 iodide (491/509), 1 mM solution in DMSO, 200 µL.
2. **DNase-free TE-buffer**
   From SIGMA (T9285-100mL); provided as 100 × Concentrate, 0.2 µm filtered, 1.0M Tris ? HCl, pH ˜8.0 containing 01. M EDTA.
3. **?-DNA**
   From New England Biolabs; provided as a standard solution of 500 µg/mL.
4. **50-mL plastic beaker**
   From Fisher Scientific.

Procedure.

Wear gloves when working with DNA. Work in the dark to prevent the dyes from bleaching. Use plastic containers for the following procedure, as the fluorescent dye may adsorb to glass. Prepare 10 mL of DNase-free TE-buffer, 10 mM Tris ? HCl, 1 mM EDTA (pH ˜ 7.5-8.0) by diluting 0.1 mL of 100 × TE buffer from SIGMA in 9.9 mL ultrapure water. Use the diluted TE-buffer to prepare a 5 µg/mL solution of ?-DNA of the biotinylated DNA working solution (carried over from the previous step). The correct proportions can be calculated once the volume of biotinylated DNA solution is known. Prepare a solution with the desired basepair to dye molecule ratio:

Basepair:Dye Molecule 2:1. To prepare a solution with 2 basepairs per staining molecule add 2.54 mL of the 5 µg/mL DNA solution to 10 µL of 1 mM YOYO-1 dye (the YOYO-1 is supplied as 1 mM, so the 10 µL can be taken directly form the container). Shake gently and let the solution rest for at least 30 minutes. This will yield 2.64 mL of 5-µg/mL fluorescent DNA solution.

Calculations: 2.54 mL 5µg/mL DNA contains 12.7 µg DNA. 10 µL 1 mM YOYO-1 contains 10^{-8} mol. Therefore, 10^{-8} mol ´ 1270.65 gmL^{-1} (molecular weight of YOYO-1) = 12.7 µg YOYO-1.

Since the DNA has a molecular weight of 649.5 gmL^{-1} of basepair and the YOYO-1 has a molecular weight of 1270.65 gmL^{-1}, the resulting solution will have 1.96 basepairs per staining molecule.

Basepair:Dye Molecule 5:1. To prepare a solution with 5-6 basepairs per staining molecule, create a ten-fold dilution (0.1 mM) of the YOYO-1 solution by adding 10 µ 1 mM YOYO-1 to 90 µL TE buffer. Add 2.54 mL of 5µg/mL DNA solution to 40 µL of 0.1mM YOYO-1. Shake gently and let rest for at least 30 minutes.

To Stain with YOYO-3 Dye (612/631):

Materials.

1. **YOYO-3 Dye**
   From Molecular Probes (Y3606); provided as YOYO-3 iodide (612/631), 1 mM solution in DMSO, 200 µL.
2. **DNase-free TE-buffer**
   From SIGMA (T9285-100mL); provided as 100 × Concentrate, 0.2 µm filtered, 1.0M Tris ? HCl, pH ˜ 8.0 containing 01. M EDTA.
3. **?-DNA**
   From New England Biolabs; provided as a standard solution of 500 µg/mL
4. **50-mL plastic beaker**
   From Fisher Scientific.

Procedure.

Wear gloves when working with DNA. Work in the dark to prevent the dyes from bleaching. Use plastic containers for the following procedure, as the fluorescent dye may adsorb to glass. Prepare 10 mL of DNase-free TE-buffer, 10 mM Tris ? HCl, 1 mM EDTA (pH ˜ 7.5-8.0) by diluting 0.1 mL of 100 × TE buffer from SIGMA in 9.9 mL ultrapure water. Use the diluted TE-buffer to prepare a 5 µg/mL solution of ?-DNA of the biotinylated DNA working solution (carried over from the previous step). The correct proportions can be calculated once the volume of biotinylated DNA solution is known. Prepare a solution with the desired basepair to dye molecule ratio:

Basepair:Dye Molecule 2:1. To prepare a solution with 2 basepairs per staining molecule, add 2.6 mL of 5-µg/mL DNA solution to 10 µL of 1 mM YOYO-3 (YOYO-3 is provided as 1 mM, so the 10 µL can be taken directly from the container). Shake gently and let the solution rest for at least 30 minutes. This will yield 2.7 mL of 5µg/mL fluorescent DNA solution.

Calculation: 2.6 mL 0f 5 µg/mL DNA contains 13 µg DNA. 10 µL 1mM YOYO-3 contains 10^{-8} mol. Therefore, 10^{-8} mol $\times$ 1322.73 gmL^{-1}(molecular weight of YOYO-3) = 13 µg YOYO-3.

Since the DNA has a molecular weight of 649.5 gmL^{-1} of basepairs and the YOYO-3 has a molecular weight of 1322.73 gmL^{-1} (1322.73 / 649.5 ~ 2), we see that the same mass creates the required solution of 2 basepairs per staining molecule.

Basepair:Dye Molecule 5:1. To prepare a solution with 5-6 basepairs per dye molecule (recommended in some cases to avoid precipitation of complexes), create a ten-fold dilution (0.1mM) of the YOYO-3 solution by adding 10 µL of 1 mM YOYO-3 to 90 mL TE buffer. Add 2.6 mL 5µg/mL DNA solution to 40 µL 0.1 mM YOYO-3. Shake gently and let solution rest for at least 30 minutes.

Basepair:Dye Molecule 3:1. To prepare a solution with 3-4 dye molecules per basepair, first prepare a 2 µg/mL DNA solution by adding 50 µL DNA standard (500 µg/mL from New England BioLabs) to 12.45 mL TE buffer. Add 1 mL 2 µg/mL DNA solution to 10 µL 1 mM YOYO-3.

IV. Attach DNA to streptavidin-coated beads [14,26].

In order to attach as many beads as possible to the biotinylated DNA molecules, we need to ensure that there is slightly more biotin (in the form of biotinylated DNA molecules) than streptavidin (coated onto the beads). If we used more streptavidin than biotin, there would be an increased chance of having beads with no DNA attached, and this would be an impediment to the overall experiment.

Remove the NaN_3 from the beads:

The Dynabeads M-270 Streptavidin are supplied as a suspension dissolved in phosphate buffered saline (PBS) pH 7.4, containing 0.02% sodium azide (NaN_3) as a preservative. This preservative should be washed from the beads before adding another buffer.

Materials.

1. **Dynabeads M-270 Streptavidin**
   Purchased from Dynal Biotech, supplied as a 10 mL (~50 mg/mL) suspension dissolved in phosphate buffered saline (PBS) pH 7,4, containing 0.02% NaN_3 as a preservative.
2. **Magnet for washing the beads**
   Have in lab.
3. **Pipette**
   Have in lab.

Procedure.

Determine the amount of beads needed for optimal binding of the biotinylated DNA[5]. Use a magnet to facilitate the removal of the suspension liquid. Shake the vial containing the beads gently to resuspend the beads in a homogeneous suspension. Add more than the calculated amount of the bead solution to a tube. This amount should be

enough to ensure that we have enough beads to attach to the DNA after washing and resuspending them. Place the tube on the magnet for 1 to 2 minutes to allow the beads to gravitate to the bottom of the tube. Do not remove the magnet during the separation process. While the tube remains on the magnet, remove the supernatant by aspiration with a pipette. Do not touch the inside wall of the tube with the pipette, as this is where the beads will be attracted to the magnet.

Resuspend the Dynabeads M-270 Streptavidin in RNase-free solution:

The Dynabeads are not supplied in RNase-free solution, so it is necessary to clean the suspension before working with DNA and/or RNA applications.

Materials.

1. **Dynabeads M-270 Streptavidin**
   Purchased from Dynal Biotech and washed free of the 0.02% NaN_3 added as a preservative.
2. **Diethylpyrocarbonate (DEPC)**
   Purchased from Fisher Scientific, supplied as 97% Diethylpyrocarbonate, 25 g.
3. **Solution A: DEPC-treated 0.1 M NaOH, DEPC-treated 0.05 M NaCl**
4. **Solution B: DEPC-treated 0.1 M NaCl**

Procedure.

Add DEPC to Solution A and Solution B to a final concentration of 0.1% (1mL/L). Shake vigorously. Incubate at room temperature for 1 hour and autoclave the solutions. Wash the beads twice with the same volume of Solution A for 1 to 3 minutes. Wash the beads once with the same volume of Solution B. Resuspend the beads in Solution B.

Resuspend the Dynabeads M-270 Streptavidin in desired buffer:

To facilitate the experiment, it is necessary to resuspend the beads in a buffer that has been used in previous steps of the experiment.

Materials.

1. **Dynabeads M-270 Streptavidin**
   Purchased from Dynal Biotech, washed free of the 0.02% NaN_3 added as a preservative and resuspended in RNase-free solution.
2. **Magnet for washing the beads**
   Have in lab.
3. **DNase-free TE-buffer**
   From SIGMA (T9285-100mL); provided as 100 ´ Concentrate, 0.2 µm filtered, 1.0M Tris ? HCl, pH ˜ 8.0 containing 01. M EDTA.
4. **Pipette**
   Have in lab.

Procedure.

Prepare 40 mL of DNase-free TE-buffer, 10 mM Tris ? HCl, I mM EDTA (pH ˜ 7.5-8.0) by diluting 0.4 mL of 100 × TE buffer from SIGMA in 39.6 mL ultrapure water. Use a magnet to facilitate the removal of the suspension liquid. Shake the vial containing the beads gently to resuspend the beads in a homogeneous suspension. Add the washed beads (in RNase-free buffer) to a tube. Place the tube on the magnet for 1 to 2 minutes to allow the beads to gravitate to the bottom of the tube. Do not remove the magnet during the separation process. While the tube remains on the magnet, remove the supernatant by aspiration with a pipette. Do not touch the inside wall of the tube with the pipette, as this is where the beads will be attracted to the magnet. Remove the tube from the magnet. Add the recommended buffer along the inside of the tube where the beads are collected. Use the same volume as used to wash the beads; resuspend gently. Repeat the separation process and removal of supernatant. After the last wash add a suitable volume of the recommended buffer to obtain an appropriate working concentration of Dynabeads.

Attach the biotinylated ?-DNA to the Dynabeads M-270 Streptavidin:

Materials.

1. **Dynabeads M-270 Streptavidin**
   Purchased from Dynal Biotech, washed free of the 0.02% NaN_3 added as a preservative, washed free of RNase, and resuspended in DNase-free TE-buffer.
2. **Biotinylated ?-DNA**
   From step II. of the experiment.
3. **Magnet for separating the beads**
   Have in lab.

Procedure.

Add the biotinylated DNA to the calculated volume of Dynabeads M-270 Streptavidin solution. Incubate at room temperature using gentle rotation or occasional mixing by gently tapping the tubes. The optimal incubation time depends on the length of the nucleic acid bound. DNA fragments up to 1 kb require 15 minutes. The ?-DNA used in this experiment has 48.5 kbp, so, presumably, it will need significantly longer incubation time to bind with the streptavidin-coated particles[6]. Separate the beads, now coated with the biotinylated DNA, using a magnet. Leave the tube containing the beads on the magnet for 1-2 minutes. Wash 2-3 times with a 1× Binding & Washing buffer (the suggested B&W buffer is 2 × concentrated, and is described as follows: 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 2.0 M NaCl). Resuspend to the desired concentration.

[1] The concentration of DNA that we have is much smaller than the 1 μM that the procedure calls for. This may affect the experiment in one of two ways: (1) the larger amount of biotin will overflood the DNA, possibly causing the reaction to proceed at a faster rate, or (2) the biotin will take a longer time to find the DNA, possibly causing the reaction to proceed at a slower rate.

2 It is unknown at this point (before experimentation with the biotin labeling kit) how much of the sample solution will remain after centrifuging and saving the aqueous phase. This volume will also most likely decrease in the next step (removing the unbound biotin).

3 The instructions for the *Biotin 3' End DNA Labeling Kit* include several procedures for estimating labeling efficiency. I am reluctant to do this, because I think it may waste much of our sample, and we need the maximum amount of biotinylated DNA to carry forward to the next steps. In addition, it should not be necessary to estimate labeling efficiency, as we should be able to determine experimentally whether the biotin successfully attached to the DNA. Therefore, I am intentionally omitting this step, however, it can easily be added back in if deemed necessary. The extra step would involve preparing a control system and checking for labeling efficiency by dot blot using either a dot/slot blotting apparatus or hand spotting.

4 At this point, it is necessary to determine the volume of sample that will be carried forward to the next step. This data will be used to calculate how much fluorescent dye is needed in the next step. According to the literature for the *Dimeric Cyanine Nucleic Acid Stains (YOYO-1 and YOYO-3)*, the optimal basepair:dye molecule ratio is at least 5:1. For the PicoGreen dye, the literature is less specific as to basepair:dye ratio, but does specify that a 2 µg/mL DNA working solution should be used.

5 Ideally, we should have slightly more DNA (biotin) than beads (streptavidin) to ensure that every bead has a DNA molecule attached to it. According to the product literature **[4]**, one milligram of the Dynabeads M-270 Streptavidin binds 700 pmol of free biotin.

Assuming that biotin attached to DNA will behave similar to free biotin, and assuming that every DNA molecule has one biotin molecule attached to it, then one milligram of beads will bind 700 pmol of biotinylated DNA. Once the volume of biotinylated DNA is known (measured after experimentation), then the number of pmol of DNA can be determined mathematically:

$$\frac{XX\ \mu L}{} \left| \frac{1\ mL}{1000\ \mu L} \right| \frac{500\ \mu g}{1\ mL} \left| \frac{1\ g}{10^6\ \mu g} \right| \frac{1\ mol}{31.5 \times 10^6\ g} \left| \frac{10^{12}\ pmol}{1\ mol} \right. = \#\ of\ pmol\ of\ DNA$$

Amount of DNA	*Conversion*	*DNA is provided as 500 µg/mL*	*Conversion*	*MW of DNA*	*Conversion*

Use the number of pmol of DNA to determine how many beads to use.

Sample Calculation: Suppose it is determined that there are 0.74 pmol of biotinylated DNA.
1 mg beads binds 700 pmol of free biotin, so 1 µg of beads will bind 0.7 pmol of free biotin (700/1000). The beads are supplied as 50 mg/mL, so that 1 µg of beads is equivalent to 0.02 µL of the beads in solution (50 mg/1 mL = 50000 µg/1000 µL = 1 µg/0.02 µL). Therefore, 0.02 µL of the beads in solution is needed for the experiment, so wash and prepare more beads than necessary to ensure that you will have enough clean beads.

6 The binding capacity of the Dynabeads M-270 Streptavidin depends on the length of the DNA. Salt concentrations also affect binding capacities. The literature states that the "optimal binding conditions for biotinylated DNA fragments (up to 1 kb) are achieved at 1 M NaCl (final concentration), 25ºC and 15 minutes". The ?-DNA molecules are entire molecules having 48502 basepairs (48.5 kb), so there may be difficulties in attaching the DNA to the beads. If we have trouble, we may need to use Dynabeads kilobaseBINDER Kit (www.dynal.no), which is designed to increase binding capabilities for longer DNA fragments.

Bibliography

1 "Avidin, Streptavidin, NeutrAvidin and CaptAvidin Biotin-Binding Proteins and Affinity Matrices." Molecular Probes. 17 May 2004. <www.probes.com/handbook/sections/0706.html>.

2 Beery, Catherine C. and Adam S.G. Curtis. "Functionalisation of Magnetic Nanoparticles for Applications in Biomedicine." Journal of Applied Physics D: Applied Physics 36 (2003): R198-R206.

3 "Biotin 3' End DNA Labeling Kit." Pierce Biotechnologies. 13 May 2004. <http://www.piercenet.com/Objects/View.cfm?type=ProductFamily&ID=06010705>.

4 Biswal, S.L. and Gast, A. P. "Mechanics of Semiflexible Chains Formed by Poly(ethylene glycol)-linked Paramagnetic Particles" Physical Review E, 68 (2), Art No. 021402, Part 1 Aug 2003.

5 "Cell Membrane–impermeant Cyanine Nucleic Acid Stains." Molecular Probes. 17 May 2004. <www.probes.com/handbook/tables/0380.html>.

6 "Components of Avidin-Biotin Technology." Pierce Biotechnologies. 10 May 2004. <http://www.piercenet.com/files/components.pdf>.

7 Cosa, G., et al. "Photophysical Properties of Fluorescent DNA-dyes Bound to Single- and Double-Stranded DNA in Aqueous Buffered Solution." Photochemistry and Photobiology. 2001. 73(6): 585-599.

8 Cremers, Daniel. "Improvement of the Procedure of Staining Confocal DNA Cytometry Specimens." June 2003. <http://www.leerhelp.nl/biomed/opdrachten/tentamen/3dstage.pdf>.

9 "Detection of DNA from Solution with PicoGreen dsDNA Quantitation Reagent in the Microplate Format." Thermo Labsystems. 18 May 2004. <http://www.pathtech.com.au/files%2F0S1Z0U7U9W9U2P9I162K2T9A2O19%2FFluorometricDetectionDNA.pdf>.

10 "DNA." Image. Tokyo Medical and Dental University. 18 May 2004. <http://www.tmd.ac.jp/artsci/ biol/textbook/chrm2dna.gif>.

11 "DNA Structure and Replication." Pearson Prentice Hall. 19 May 2004. <http://www.phschool.com/science/biology_place/biocoach/dnarep/intro.html>.

12 "DNA Quantitation Using PicoGreen Dye." BioProtocol.com. 18 May 2004. <http://www.bio.com/ protocolstools/protocol.jhtml?id=p87>.

13 "Instructions: Biotin 3' End DNA Labeling Kit." Pierce Biotechnologies. 13 May 2004. <http://www.piercenet.com/files/ACF5BA.pdf>.

14 "Instructions: Dynabeads M-270 Streptavidin." Dynal Biotech. 7 June 2004. <http://www.dynal.no/kunder/dynal/DynalMMA401.nsf/lupgraphics/353_02_13_653.0_06_Dynabeads_M270_Streptavidin.pdf/$file/353_02_13_653.05_06_Dynabeads_M270_Streptavidin.pdf>.

15 "Instructions: Slide-A-Lyzer MINI Dialysis Unit." Pierce Biotechnologies. 27 June 2004. <http://www.piercenet.com/files/0803as4.pdf>.

16 "Introduction to Fluorescence Techniques." Molecular Probes. 17 May 2004. <http://www.probes.com/media/pis/mp07581.pdf >.

17 "Introduction to Optical Tweezers." Niels Bohr Institute. 17 May 2004. <http://www.nbi.dk/~tweezer/introduction.htm>.

18 Marie, Dominique, Danile Vaulot, and Frederic Partensky. "application of the Novel Nucleic Acid Dyes YOYO-1, YO-PRO-1, and PicoGreen for Flow Cytometric Analysis of Marine Prokaryotes." Applied and Environmental Microbiology. May 1996. 62: 1649-1655.

19 "Material Safety Data Sheet: Tris Buffer." Proscitech.com. 18 May 2004. <http://www.proscitech.com.au/catalogue/msds/c021.pdf>.

20 "Nucleic Acid Stains." Molecular Probes. 18 May 2004. <http://www.probes.com/handbook/sections/0801.html>.

21 Pankhurst, Q.A., J. Connolly, S.K. Jones, and J. Dobson. "Applications of Magnetic Nanoparticles in Biomedicine." Journal of Physics D: Applied Physics 36 (2003): R167-R181.

22 Perkins, Thomas. "Gallery Photograph: relaxation of 39 µm-long DNA molecule." Molecular Probes. 18 May 2004. <http://www.probes.com/servlets/photo?fileid=g000465>.

23 "Photophysical properties of fluorescent DNA-dyes bound to single- and double-stranded DNA in aqueous buffered solution." Photochem Photobiol. June 2001. <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=11421063&dopt=Abstract>.

24 "Product Information: Dimeric Cyanine Nucleic Acid Stains." Molecular Probes. 17 May 2004. <http://www.probes.com/media/pis/mp03600.pdf>.

25 "Product Information: PicoGreen dsDNA Quantitation Reagent and Kits." Molecular Probes. 17 May 2004. <http://www.probes.com/media/pis/mp07581.pdf>.

26 "Products: Dynabeads M-270 Streptavidin." Dynal Biotech. 7 June 2004. <http://www.dynal.no/kunder/dynal/dynalpub401.nsf/($All)/841E0473B0D7A8FAC1256C5400488DF4?OpenDocument>.

27 Promega, Doug. "Q & A Technically Speaking: Streptavidin MagneSphere Paramagnetic Particles." PromegaNotes. 17 May 2004. <http://www.promega.com/pnotes/66/7014_17/7014_17_core.pdf>.

28 Schäfer, Bürk, Helgard Gemeinhardt, Volker Uhl, and Karl Otto Greulich. "Single Molecule DNA Restriction Analysis in the Light Microscope." Single Molecules 1 (2000) 1: 33-40.

29 "Slide-A-Lyzer MINI Dialysis Unit." Pierce Biotechnologies. 27 June 2004. <http://www.piercenet.com/Objects/View.cfm?type=ProductFamily&ID=04010165>.

30 Song, Gongwu, et al. "Fluorometric Determination of DNA Using a New Ruthenium Complex $Ru(bpy)_2PIP(V)$ as a Nucleic Acid Probe." Analytical Sciences. July 2002. 18: 757-759.

31 "Standard Operating Procedure: PicoGreen Working Solution Preparation for DNA Quantification." Sequenom, Inc. 18 May 2004. <http://medicine.ucsd.edu/hypertension/list_of_protocols/35_Picogreen%20Working%20Sol.pdf>.

32 "The Basic Structure of DNA." AllScienceStuff.com. 18 May 2004. <http://www.allsciencestuff.com/mbiology/concepts/dna>.

33 "The Picofluor™ Method for DNA Quantitation Using PicoGreen®." Molecular Probes. 24 May 2004. <http://www.thelabrat.com/protocols/DNA-RNAQuantitation7.shtml>.

34 "Visualisation of Ligand-Protein Interactions." University of Cambridge Department of Pharmacology. 18 May 2004. <http://www.phar.cam.ac.uk/RI/AFM/LP.html>.

35 Wirtz, Dennis. "Direct Measurement of the Transport Properties of a Single DNA Molecule." Physical Review Letters 75.12 (1995): 2436-2439.

36 Zhu, Chang-Qing, et al. "Determination of Nucleic Acids by Near-Infrared Fluorescence Quenching of Hydrophobic Thiacyanine Dye in the Presence of Triton X-100." Analytical Sciences. June 2004. 20: 945-949.