APPLICATION NOTE

No. T091

Using the NanoDrop 3300 Fluorospectrometer

For micro-volume fluorescence/FRET applications

Introduction

FRET (Fluorescence Resonance Energy Transfer) is the transfer of excited-state energy from the initially excited donor fluorophore (D) to an acceptor fluorophore (A). The spectral overlap between the emission of D and the absorbance spectrum of A and the physical distance between the two are important factors in determining the efficiency of energy transfer.[1,2]

The Thermo Scientific™ NanoDrop™ 3300 Fluorospectrometer uses a patented "cuvette-less" sample retention system for fluorescent measurements using as little as one microliter sample volume. With UV, blue and white LED excitation, it covers a broad wavelength range, thereby facilitating the use of many diverse fluorophores; it is also able to analyze multiple emission profiles from a single sample. The following examples describe some of the uses of NanoDrop microvolume fluorescence technology as it pertains to FRET research.

DNA methylation

DNA methylation is an important regulator of gene transcription, and the role of DNA methylation in carcinogenesis has been an increasing area of focus in the field of epigenetics over the last decade. Alterations in DNA methylation patterns are common in a variety of tumors and also play an essential role in cellular development. Methylation sensitive PCR (MSP) is a commonly used technique to assay for methylation detection. MSP reactions consist of several cycles of PCR and may require an additional round of amplification.

Recently, a new technique, methylation-specific quantum dot fluorescence resonance energy transfer (MS-qFRET), has been developed. MS-qFRET is a faster and more sensitive technique than MSP for detecting minute amounts of methylated DNA in the presence of a 10,000 fold excess of unmethylated alleles. In order to measure fluorescent probes, scientists often use high- end spectrofluorometers, which can be very expensive and complicated, or resort to relatively awkward and unreliable lab-built set-ups using microscopes and CCD cameras. In a progressive approach, Bailey *et al.* successfully used the NanoDrop 3300 Fluorospectrometer to measure QD 605-Cy5 FRET probes in proof of concept experiments, as well as in the method development and implementation of the new technique.[3, 4]

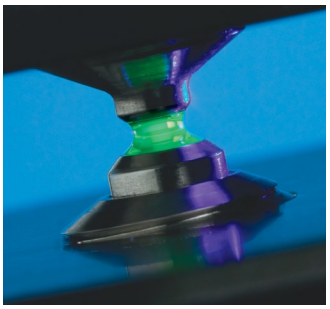

Figure 1: Close up view of the sample retention system of the NanoDrop 3300 with a concentrated sample of FITC forming a column between the measurement pedestals.

Protein-DNA quadraplexes

Guanine rich nucleic acid sequences have been reported to form secondary structures, stabilized via Hoogsteen hydrogen bonding, recognized as G-quadruplexes. These quadruplexes have been hypothesized to influence transcription of proto- oncogenes, ultimately leading to carcinogenesis. Kumar *et al.* used dual labeled oligonucleotides with the common FRET pair FITC-TAMRA in kinetic experiments to determine structural interactions of DNA with polyamines in a c-MYC quadruplex model.[5] The micro-volume capability of the NanoDrop 3300 Fluorospectrometer sample retention system allowed for kinetic measurements of a single reaction without the need to transfer the sample to a cuvette or other containment device for measurement. Their work is helping to elucidate the role polyamines play in DNA transcriptional activation.[5]

Multi-labeled DNA quadruplexes for pattern based protein detection have also been constructed by Margulies and Hamilton.[6] Their work resulted in a homogenous solution based detection system using four unique fluorophores in a combinatorial sensory array, for detection of select proteins in microliter sized reactions. Current development efforts in pattern based detection have consisted of either the use of solid state supports or measurements in non-homogeneous liquid samples. A major limitation of the current chip based methods by which these measurements are taken is the large quantity of sample required and effort that is needed to manufacture solid state arrays. Proof of concept experiments utilizing custom synthesized fluorescently labeled G-quadruplex protein sensors in micro-volume reactions were validated utilizing the sample retention technology of the NanoDrop 3300 Fluorospectrometer. This sensing process has potential applications in medical diagnosis, pathogen detection and proteomics.[6]

Summary

The NanoDrop 3300 Fluorospectrometer is well suited to measure multiple fluorophores in homogenous samples and may be used for FRET based assays, including those where sample size is minimal. Endpoint analysis as well as workflow applications are possible without the need for instrument warm-up, cuvettes, or filter changes. The NanoDrop 3300 software includes pre-programmed methods for measuring a variety of common fluorophores, as well as a Method Editor, which provides the capability of building custom methods for specific FRET pairs (Figure 2). The diversity of fluorophores (e.g., FITC, TAMRA, Q-dot 605, Cy5 and Pyrene) that have been used in conjunction with the NanoDrop 3300 Fluorospectrometer highlights the utility of the three LED excitation sources and the broad emission range of the instrument. Lower overall costs result from reduced operator time, lack of disposables, and very small sample requirements. The entire cycle time, which includes dispensing one microliter of sample onto the optical surface, measuring its fluorescence, and blotting the optical surface for the next sample, is commonly completed within 30 seconds.

Another advantage of the instrument is its portability and small footprint which conserves precious laboratory bench space. The instrument is powered through a USB connection to a computer, on which all measurement data is archived. Based on these studies, the NanoDrop 3300 Fluorospectrometer has proven to be a valuable, cost-effective tool for FRET based protocols.

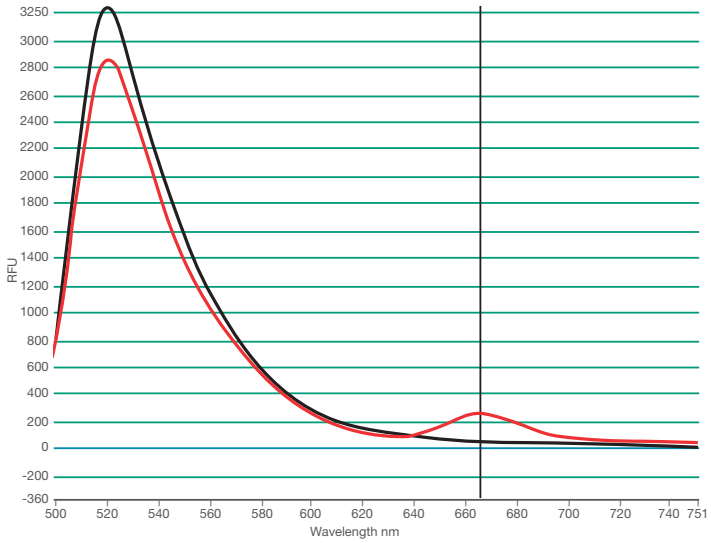

Figure 2: Fluorescence emission spectra of a FITC-Cy5 FRET oligonucleotide with complementary target sequence present (red) and absent (black) displayed in the NanoDrop 3300 data viewer software.

References

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6. Margulies, D., Hamilton, A. Protein Reconition by an Ensemble of Fluorescent DNA G-Quadruplexes. *Angew Chem Int Ed Engl.* **2009**. (10):1771-1774.

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