BIOPTIX ANALYTICAL

404Pi™

A Simple Study of Real-Time Oligonucleotide Duplex Formation and Dissociation

Introduction

Oligonucleotide duplex formation and dissociation represents a useful model system for studying macromolecular interactions. Formation and dissociation of these duplexes can be observed in real time using the BiOptix 404Pi™. Measurements of this sort are of practical use in studying microRNA sequence variations and genetic mutations such as base substitutions, deletions, and duplications.

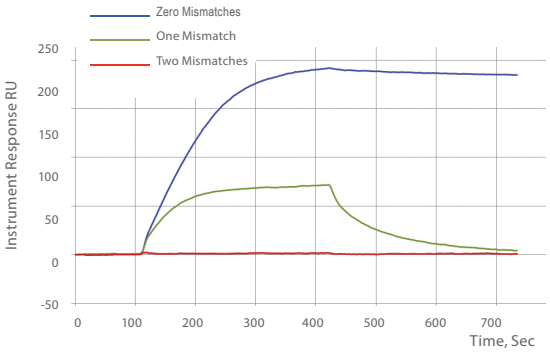

Figure 1.
Sensorgrams of oligonucleotide duplex formation and dissociation for zero, one and two sequence mismatches.

Materials and Methods

All oligonucleotides were purchased from IDT-DNA Technologies. Biotinylated (capture) oligonucleotides (100 nM in PBS) were applied to a BiOptix SA-150™ (Streptavidin) SensorChip while in the instrument at a flow rate of 60µL/min for 5 min. Non-biotinylated ("target") oligonucleotides (100 nM) were sequentially injected into the flow cell, exposing them to the capture oligonucleotide. Regeneration was accomplished using a single 20 sec injection of 20 mM NaOH.

Results

This technique has proven to be very sensitive to changes in the sequence of synthetic oligonucleotides. One example involves detecting mismatches within a 12-mer oligonucleotide duplex. Figure 1 shows the result of such an experiment. A 12-mer biotinylated oligo (Biot1) was loaded onto a BiOptix SA-150™ SensorChip while in the instrument. Sol1 (exact complement to Biot1), Sol2 (one mismatch), and Sol3 (two mismatches) were then sequentially injected with intermediate regenerations. The sensitivity of this assay at discriminating the number of mismatches within a pair of oligonucleotides is shown in Figure 1. Sol3 showed no apparent binding to Biot1 while Sol2 showed a reduced affinity for Biot1 as compared to Sol1.
It should also be noted that DNA-DNA hybridization kinetics are quite complicated and under the conditions of this experiment do not fit a simple 1:1 binding model.

Discussion

These experiments demonstrate the utility of the BiOptix 404Pi™ in studying the formation and stability of oligonucleotide duplexes.