Project 3: "Development of a One-Step RNA Virus Detection System"

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Project Summary

The Norwalk viral agent or norovirus is the most prevalent cause for non-bacterial gastroenteritis worldwide [1-4] and studies by the Centers for Disease Control (CDC) indicate that 1 in 10 Americans will contract acute gastroenteritis each year as a result of norovirus infection [5]. Norovirus has been detected in river water, tap water, seawater, and mineral water [6], making it a waterborne pathogen of concern, particularly for developing areas. Due to the low infectious dose, highly sensitive methods are required to accurately detect norovirus contamination. The standard detection method for norovirus is reverse transcriptase-quantitative PCR (RT-qPCR) which can detect <10 transcript copies [7] . In addition to the time and equipment requirements of this method, it can also be prone to false negatives [8] and require multiple tests to comprehensively test for all norovirus strains [9]. The detection of norovirus is also hindered by factors including inappropriate sample storage, low virus concentrations and ineffective

viral RNA extraction methods [10], particularly for surface waters. Previous studies have found that duplex specific nuclease (DSN), originally from the Kamchatka crab [11], has previously been used for fluorescent detection of microRNAs, as shown in Figure 1 [11-13], but detection is limited by a preference for short targets (<20bp) [14, 15]. The teachers will be creating mutant versions of the DSN enzyme to increase nuclease activity on longer RNA targets. The mutated DSN enzymes will be combined with Taqman probes designed to hybridize to specific regions of the norovirus genome to create a single step viral detection assay. The primary goal for this project is to examine the full range of DSN functionality through single amino acid mutations. An improved DSN enzyme would advance viral detection methods, allowing for faster, more accurate detection and subsequent treatment.

Possible Ideas for Classroom Implementation

Teachers will be trained in relevant molecular biology techniques needed for DNA mutation, protein purification and analysis, and statistical interpretation of the results in Excel and Origin. Given the framing of this project in terms of water quality and waterborne disease, teachers could link this project to units on water characteristics and quality. Biology units could explore protein folding and structures to discuss the impact of mutations on protein function. Teachers and students may be invited to tour the AC/SAC labs or the AC/SAC may visit classrooms to provide demonstrations.

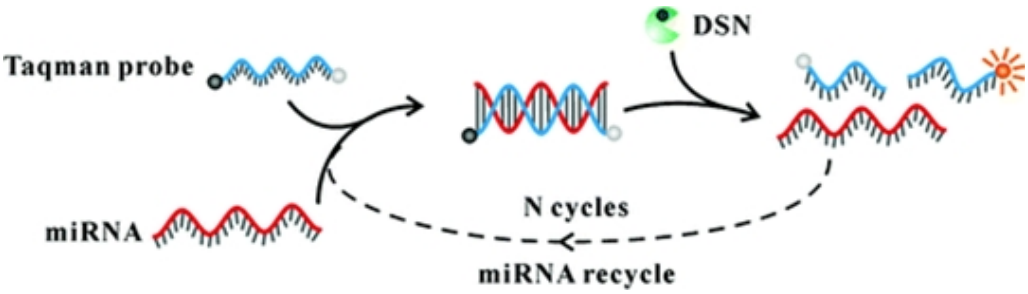

Figure 1: DSN Cleaves the TaqMan Probe to Produce Fluorescence [12]

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