

Study of Coronavirus Protease Using CFP-YFP Fluorescent Assay

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Middle Eastern Respiratory Syndrome (MERS) is an emerging viral disease originating in the Arabian Peninsula with a current mortality rate of nearly fifty percent throughout Europe and Asia according to the World Health Organization. Characterization of this disease is being done to understand the basis of viral replication. One target for viral inhibition are replication proteases. Replication proteases are enzymes that cleave proteins specific to cell growth and reproduction that form the viral replicase complex making them an ideal target for viral replication inhibition. First, replication proteases were characterized using a fluorescence resonance energy transfer (FRET) construct by measuring the amount of fluorescence emitted during enzymatic activity. This construct produces a measurable change in fluorescent activity to analyze the rate at which replication proteases cleave proteins essential for viral growth. Once this assay was completed, data was extracted and enzymatic kinetic calculations were performed to continue further analysis of enzymatic activity. The results produced from these experiments will allow a comparison of replication proteases specific to MERS with other viral replication proteases. Further analysis will be done to measure varying cleavage rates of different coronaviruses. This study produces conclusive results for the characterization of MERS replication proteases that are essential in further development of inhibitor molecules.