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Dengue RdRp characterization

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Dengue virus kills 25,000 people annually in affected tropical regions. Non-structural protein 5 of the virus has RNA-dependent-RNA-polymerase and methyltransferase activity in separate domains. Since genome duplication is essential for viral replication, RdRp is an attractive target for drug design. Additionally, the four Dengue virus serotype ns5 proteins have a 67% shared amino acid identity, suggesting that discovered drugs may engage in cross-inhibition. We expressed and purified the polymerase domain of ns5 in an overexpressing E. Coli system. The enzyme was purified by immobilized metal affinity chromotography. This construct, ns5Pol, was found to be enzymatically active in an in vitro assay. Activity was characterized and optimal conditions were determined. Polymerase reactions with ns5Pol were shown to be dependent on salt concentration, template/primer ratio, reaction temperature and pH. Dengue ns5Pol activity appears to be suppressed by a polyoxometalate known to inhibit other viral polymerases. We are currently developing a high-throughput RNA polymerase assay to screen a library of pre-selected compounds that pass Lipinski's rule of five.