Title: Early Detection of KRAS G12D Mutation Using CRISPR-Cas12a and REA-RPA to Increase Survival Rates of Pancreatic Cancer Patients.

Abstract: Pancreatic cancer is one of the most lethal cancers with a dismal 5-year survival rate of about 13% due to diagnostic difficulties and treatment resistance. Early, affordable, and convenient detection methods are urgently needed. Diagnostics like imaging and biopsies are invasive, costly, and often inaccessible to at-risk individuals.

Our project targets the *KRAS^G12D* mutation, a key driver of pancreatic cancer, using a CRISPR-Cas12a detection system. Cas12a, guided by a custom crRNA, selectively binds mutant KRAS mRNA, activating cleavage of a fluorescently-labeled DNA reporter. Cleavage separates the fluorophore from its quencher, increasing fluorescence intensity that signals mutation presence in a fluorometric readout.

We are developing a cell-free assay combining Restriction Endonuclease Assisted-Recombinase Polymerase Amplification (REA-RPA),NlaIII enzymes to create recognition sites, and Cas12a-crRNA complexes for mutation detection. The assay is designed to be rapid and user-friendly, suitable for at-risk populations to quickly assess the need for medical consultation.

Currently, molecular components are designed, and we plan to validate construction with synthetic KRAS RNA controls. Successful development could enable early pancreatic cancer detection, improve survival, and reduce healthcare burdens. This project advances CRISPR diagnostic tools toward accessible, point-of-care cancer screening creating a detection device that works for specific mutations.