Micro RNA223-Biomarker Based Exponential Rolling Circle Amplification CRISPR -Cas12a System for Disease Detection and COPD Diagnostics

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This project implements a novel detection system based on Rolling Circle Amplification (RCA) and Cas12a for miRNA biomarkers, and provides an efficient and cost-effective approach for the diagnosis of chronic obstructive pulmonary disease (COPD). Reverse transcription real-time polymerase chain reactions (RT-qPCR) were performed as a basis for comparison. A proof-of-concept process was then provided, followed by characterization and calibration of the novel detection system. The sequence of characterization experiments demonstrate the optimality, specificity and sensitivity of the system. The reaction time is reduced to under 20 minutes, while the reagent cost per reaction is $0.6. The system responds to sequential changes of one base pair, is able to detect down to the femtomolar (fM) range, and has a more significant difference in average fluorescence compared to RT-qPCR, the standard miRNA detection method (demonstrating sensitivity). Furthermore, a Visual Based Disease Detection Device (VBD3) was created as a portable fluorescent quantifier that performs analysis and quantification on the fluorescent signals generated. In addition, the discussion section discusses the adaptability of the project for miRNA biomarker-based detection of other diseases, as well as the potential of multiplexes to ensure more comprehensive diagnosis and complementary hardware to quantify the fluorescent signal.