Genetics Day Poster Submission

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Title: ULTRASENSITIVE CAS13A-BASED DIAGNOSTIC TOOLS AND NEW DESIGN STRATEGIES FOR SINGLE-NUCLEOTIDE POLYMORPHISM DETECTION

Abstract: Molecular tools to dissect RNA function are valuable for studying the diverse roles RNA plays in cellular function, and as potential novel therapeutics, given thousands of dysregulated RNAs have been observed across a range of diseases. Recently described programmable nucleases, such as the prokaryotic CRISPR-associated nuclease Cas13, offer the potential to develop precise and flexible RNA-targeting technology. Cas13 has been successfully employed for potent and specific RNA-knockdown in eukaryotic cells, and other exciting RNA-binding applications include RNA imaging, RNA-splicing, RNA-detection or RNA-editing applications, for example. For Cas13 diagnostics, multiple efforts in recent years have focused on establishing powerful platforms that could couple nucleic acid amplification to Cas13-detection for higher sensitivity (SHERLOCK, CREST), streamline single-step protocols (SHINE), perform multiplex testing (CARMEN) or use different Cas13 orthologs (SENSR). These robust platforms are promising to be deployed at point-of-care locations making testing accessible, scalable, faster, low-cost, and flexible, which is particularly relevant in the context of fast-developing outbreaks. However, exploration of the principles for crRNA design in the context of Cas13-based diagnostics has been limited, leaving it a case-by-case basis exploration of suitable crRNAs. This is particularly relevant when deploying Cas13 to distinguish a pathogen's genetic variation or whether a sample is pathological or not. Further progress in the diagnostic field requires a deeper understanding of the biophysical parameters that underlie Cas13 RNA-recognition and activation which in turn will guide the rational design of more specific Cas13 RNA-diagnostics. By using various methods including mismatch tolerance profiling, crRNA length studies, and structure-guided engineering of Cas13a, we report new strategies and Cas13a variants that yield highly specific discrimination of RNA-targets down to single-nucleotide polymorphisms (SNP). We deployed this novel platform for the detection of single-nucleotide polymorphisms in SARS-CoV-2 variants of concern and showed its potential for disease diagnostics or epidemiological surveillance.