Utilizing a Molecular Program for the Hypersensitive Detection of Nucleic Acids

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1. Introduction

An emerging new method for detecting biomarkers, involves the profiling of circulating nucleic acids (CNA), as early-stage, minimally invasive diagnosis (2). However, detection can be challenging, because they may have very short sequences, high sequence homology and are also present in low concentrations in the blood. The tools used to profile them need to be sensitive, specific and quantitative.

2. Detection System

Here we present a molecular program in the form of an in vitro nucleic acid circuit, designed to detect specific target sequences. This system is then combined with droplet microfluidics to provide absolute quantification of the target, similarly to digital PCR. According to the Poisson law, only droplets containing the target will fluoresce, so the original target copy number in the sample can be calculated. The detection program is a self-organized dynamic system rationally designed with a standardized set of templates, enzymes and dNTPs as fuel. These components interact with each other to emit a strong fluorescent signal, in the presence of a single copy of target sequence. This detection method has several advantages over the current gold-standard RT-qPCR. It is an isothermal 1-step reaction. It is also less prone to background signaling. And combining it to droplet based microfluidics provides absolute quantification and does not require calibration or internal controls. Our results thus far show we can successfully detect synthetic targets in human plasma background (Figure).

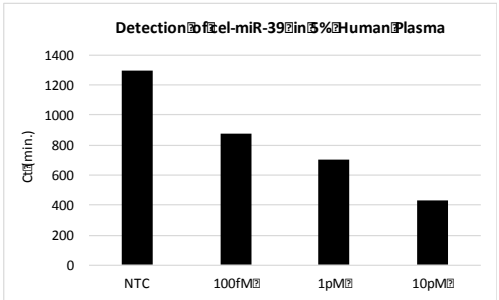

Figure: Detection of cel-miR-39 concentration range spiked in 5% human plasma extract. Ct values correspond to 0.5 normalized RFU.

References

[1] Montagne, *et al.*. Boosting functionality of synthetic DNA circuits with tailored deactivation. Nature Communications, 7, 13474 (2016)

[2] Pekin, *et al*. Quantitative and sensitive detection of rare mutations using droplet-based microfluidics. Lab on a Chip, 13 (2011)