Paper-based Device for Point-of-care Nucleic Acid Quantification Using CRISPR/Cas System and Personal Glucose Meter

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Recently, clustered regularly interspaced short palindromic repeat (CRISPR)-based assays have been reported as attractive tools for nucleic acid detection due to their high specificity and sensitivity. Taking advantage of the low cost and wide availability of personal glucose meters (PGMs), methods combining the CRISPR/Cas system and PGMs for nucleic acid quantification have been reported for point-of-care testing (POCT). They rely on the conversion of the target nucleic acid concentration into a glucose signal through an enzymatic reaction. However, most reported assays require multi-step operations involving pipetting and separation, which is against the concept of POCT.

In this work, we developed a paper-based biosensor for quantification of nucleic acids by combining the CRISPR/Cas system and PGMs. Pre-deposition of all required reagents on a multi-layer paper device enables assays to be performed by end-users without multiple operation steps and reagent handling. The device consists of three layers of hydrophobic wax-patterned paper (Fig. 1). A target DNA-specific Cas12a-CRISPR RNA (crRNA) complex and single-stranded DNA-conjugated invertase immobilized on magnetic beads (MB probe) are deposited on the first layer, while sucrose is dried on the third layer. Application of a sample containing target DNA (tgDNA) onto the first layer of the paper device activates the Cas12a-crRNA complex, resulting in release of invertase through nonspecific trans-cleavage of ssDNA at the surface of the MB probe. After the cleavage reaction, removing a hydrophobic film separating the first and second paper layers allows the released invertase to flow through the second filter layer, while magnetic beads are retained. When reaching the third layer, the released invertase converts the pre-deposited sucrose to glucose, which is subsequently detected by the PGM. A tgDNA concentration response was obtained from a single sample application (10 μL) without any further user intervention except for the hydrophobic film removal and washing buffer application (Fig. 2). For proof-of-concept, the detection of human papillomavirus-DNA (43 base pairs) has been achieved.

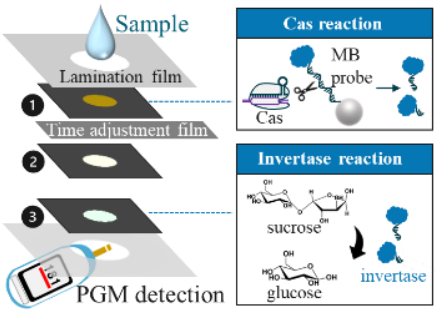

Fig. 1 Device design

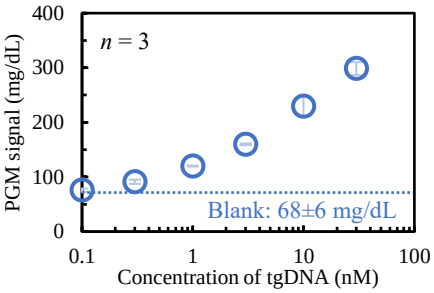

Fig. 2 Calibration curve with device