Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

A Critical Review of Microfluidic Systems for CRISPR Assays

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This document contains the following additional figures and other supplementary information:

- [S1] Review of existing CRISPR published reviews.

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There exists a significant number of reviews of the general use of CRISPR-Cas enzymes as a molecular tool. Notable examples relevant to CRISPR-based diagnostics include that of Kaminski *et al.*[1] who presented an excellent review of the history and biochemistry of CRISPR-Cas enzymes, including basic assay designs. Tang *et al.*[2] reviewed the advent of these systems and provided an excellent summary of assay strategies and technologies that use CRISPR for diagnostics. Other general CRISPR diagnostics reviews include those of Azimzadeh *et al.*[3] and Granados-Riveron *et al*.[4]

A few general reviews of CRISPR diagnostics have included some microfluidic device strategies and components. For example, Tang *et al.*[2] reviewed a few notable microfluidic devices, including lateral flow assay systems for the final signal detection. A few reviews have specifically addressed the possible role of CRISPR enzymes in point-of-care (PoC) devices, and these tend to strongly emphasize microfluidic devices. Examples of the latter include the reviews of van Dongen *et al.*[5] and Brogan *et al*.[6] Other reviews which included references to and examples of microfluidic devices include those of Ganbaatar *et al*.[7] and Palaz *et al.*[8]

To our knowledge, the only review to date to focus on microfluidic devices which use CRISPR-Cas enzymes as an assay tool is the recent work of Chen *et al*.[9] The latter review usefully categorized assays by the driving force to effect assay steps (e.g., pressure-driven flow or magnetic forces) and by their multiplexing capacity. The latter review concentrated on the possible advantages of CRISPR-based assay tools and not on their disadvantages or remaining challenges. As one important example of this, Chen did not discuss the intimate relation between assay limits of detection (LoDs) and enzymatic kinetic rates. As we shall discuss here, the kinetic rates of CRISPR remain an open question and this obscures their assessment as a diagnostic tool.[10] In fact, many (if not most) microfluidic CRISPR assays for trace analytes will require integration of pre-amplification into their workflow. The Chen review also excluded assays which use lateral flow biosensors in the readout portion.

References

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