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MULTI-SPECIES DETECTION OF Cas9 PRESENCE AND ACTIVITY ON A PORTABLE CENTRIFUGAL MICROFLUIDIC PLATFORM

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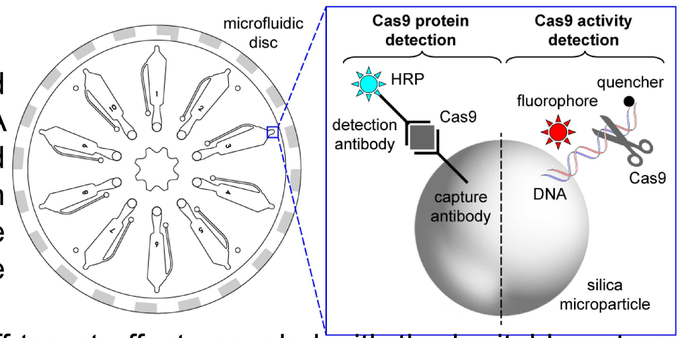
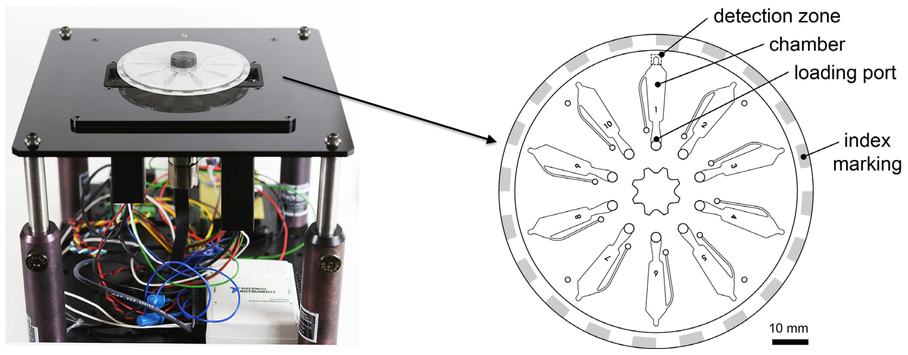
INTRODUCTION

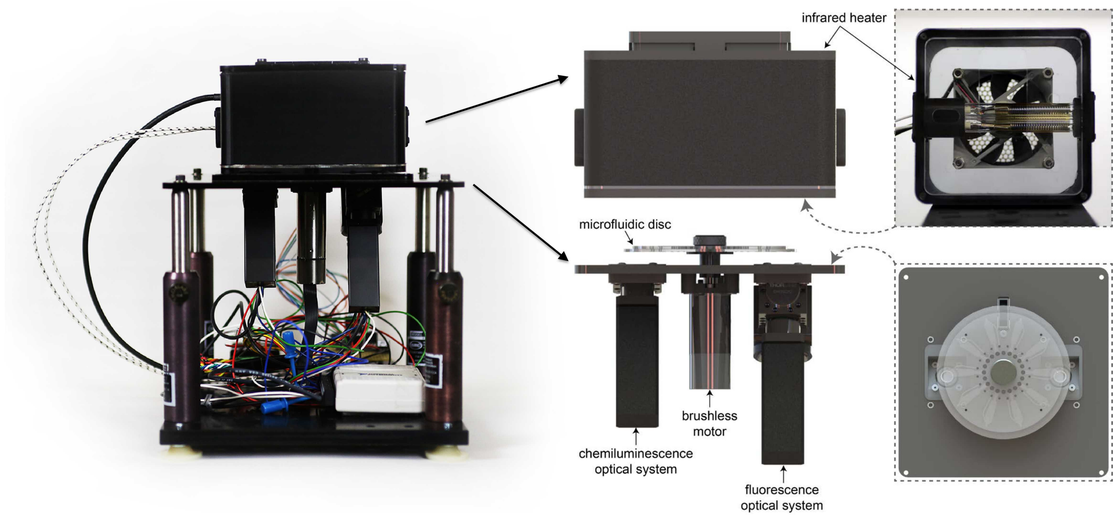


The growing use of facile, targeted gene editing by the RNA-guided DNA nuclease CRISPR-Cas9 has created an explosion of activity with implications in both basic science and applied, clinical research. While the system holds great promise for treatment of disease, potential for off-target effects coupled with the heritable nature of genetic modifications highlights the need for sensitive Cas9 detection assays. Here, we report the use of a centrifugal, microfluidic platform for the detection of both Cas9 presence and activity. Assays for the detection of the widely-used *S. pyogenes* Cas9 performed at picogram-per-milliliter sensitivity with commercially-available antibodies. Detection assays were also demonstrated for Cas9 from S. aureus, N. meningitides, and S. thermophilus. The use of phage-derived anti-CRISPR protein AcrIIC1 was shown to be an excellent capture reagent for several species of Cas9. A fluorescent sedimentation nuclease activity assay was also included to demonstrate sensitive, simultaneous detection of both Cas9 protein and activity from a single biological sample.

DETECTION PLATFORM



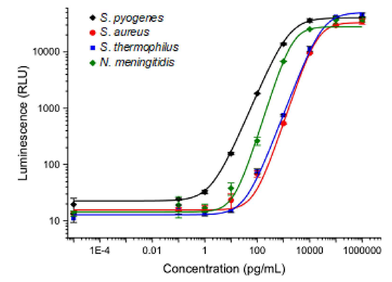
The microfluidic device at the heart of the platform is a simple three-layer disc designed to transport signal molecule-enriched microparticles from the center of the disc through a density matrix and into the detection zone located at the periphery of the disc. Cast acrylic sheets were ablated with a laser to form the structural top and bottom components held together by a pressure-sensitive adhesive layer into which the channels were cut.

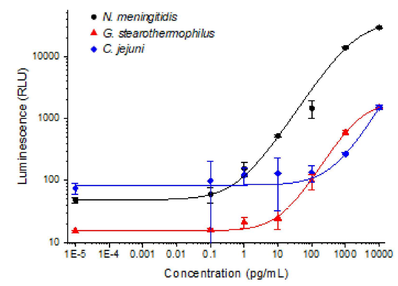


The platform features two optical detection subsystems to enable the dual reporter molecule scheme for the assays. The fluorescence optical system utilizes a red (635 nm) laser diode in an epifluorescence configuration with a photomultiplier tube at the end of the optical train. The chemiluminescence optical system has no excitation source and has the PMT directly in line with the disc. The two systems are not active at the same time to avoid crosstalk. A 3D-printed lid covers the system and prevents ambient light from interfering with measurements. The lid has an infrared emitter built into it for temperature control to ensure reproducibility.

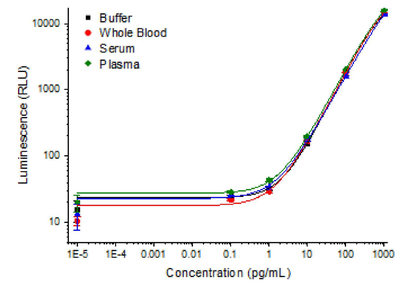
Cas9 DETECTION

Protein detection proceeds via a standard immunoassay pulldown architecture. A capture particle is decorated with affinity reagents and forms a complex in the presence of the target antigen and a detector antibody. The complex is then spun through a density medium to the tip of the channel, a process which washes, separates, and concentrates the signal into the detection region at the periphery of the disc. Four species of Cas9 were detected using commercially-available antibodies. Three species of Cas9 were detected using anti-CRISPR protein AcrIIC1. Sensitive limits of detection are reported for each of the species.

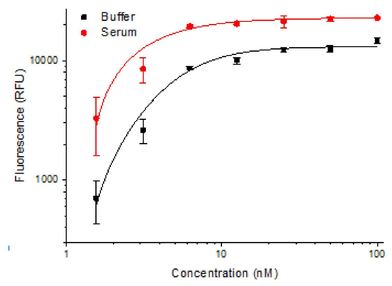




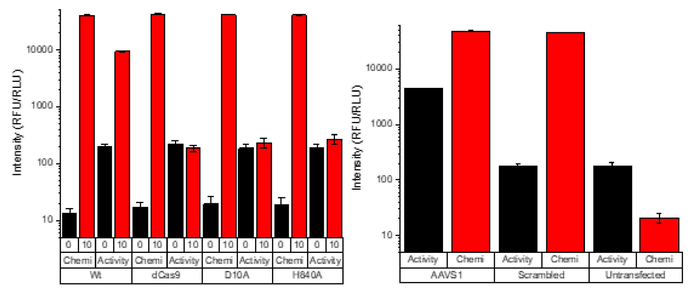
Unique to this platform is the ability to work directly from clinical sample matrices with little impact on detection limits. Here, we demonstrate detection in buffer, whole blood, serum, and plasma. The dose-response curves are nearly overlapping for each of the biological matrices shown.



In order for CRISPR/Cas9 to edit genes, it must cut the DNA functioning as a nuclease. We recently published an activity assay for multiple Cas9 species and have adapted this technology to the microfluidic platform. Results from buffer and serum show similar dynamic range and sensitivity in detection.



Demonstrated detection of Cas9 protein presence and activity from the same sample at the same time. The flexible nature of the assay and device architecture make this amenable to changing the reagents for a wide range of targets.



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