SURFIX diagnostics

WHITE PAPER

Detection of salmon pathogen using a CRISPR/dCas9 based DNA assay on a photonic biosensor

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Introduction

Aeromonas species are the most common source of bacterial infection in fish. High density of fish in aquaculture often leads to fast disease spread, massive fish death, and water contamination. Early detection of bacterial pathogens could provide better disease control. Currently, culture-based methods and DNA amplification followed by next generation sequencing (NGS) are the most widely used techniques to detect pathogens [1]. These diagnostic methods are often not available in resource-limited settings and require sophisticated infrastructure and experienced personnel.

Surfix Diagnostics has developed a photonic biosensor platform based on silicon nitride waveguide technology [2]. This platform can directly measure double stranded DNA (dsDNA) without requiring cell culture or DNA amplification. This easy-to-use, reliable, and sensitive technology enables fast detection of multiple biomarkers simultaneously. These features, along with a potentially low unit cost and the ability to easily scale up production, has the potential to revolutionize onsite testing in aquaculture. In the Horizon 2020 project PHOTO-SENS*, a demonstrator photonic biosensor system for salmon pathogen detection is being developed.

Detection of pathogen-specific dsDNA using CRISPR/dCas9 system

CRISPR/Cas-based biosensing technology has the potential to be a powerful pathogen diagnostic tool [3]. The CRISPR/Cas system consists of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) proteins. In this work, we developed a CRISPR/Cas based assay to detect DNA in vitro.

Cas9 protein from Streptococcus pyogenes together with a single guide RNA (sgRNA) provides a programmable ribonucleoprotein (RNP) complex that cleaves specific sequences of target dsDNA. dCas9 is a modified version of Cas9 nuclease, which does not have the ability to cleave dsDNA but is still able to recognize and bind target dsDNA sequences. The dCas9 system therefore allows the direct detection of dsDNA, thus avoiding the undesirable DNA denaturation and subsequent hybridization steps required in DNA assays based on detection of single stranded DNA. The present study describes the detection of bacterial dsDNA using either PCR fragments or whole genomic DNA (gDNA) on Surfix's photonic biosensor platform.

Each photonic chip comprises 6 biosensor elements configured as an array of asymmetric Mach-Zehnder Interferometers (aMZIs) [2]. First, chips were provided with Surfix's performance enhancing material-selective nanocoating [2]. Next, CRISPR/dCas9 functionalized biochips were prepared by immobilization of RNP on the waveguide surface. The dCas9 specificity was programmed by sgRNAs specific for the vapA gene of Aeromonas salmonicida (A. *sal*).

Genomic DNA samples were provided by TunaTech GmbH (Düsseldorf, Germany). The gDNA was isolated from A. sal (target DNA) and Vagococcus salmoninarum (V. *sal*, non-target DNA) and was used for PCR

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fragment generation (626 bp for A. *sal*, 337 bp for V. *sal*) or for direct measurements without DNA amplification.

Results

To determine the specificity and sensitivity of the assay, chips were incubated with samples containing different concentrations of target dsDNA (A. *sal*) or non-target dsDNA (V. *sal*). For PCR fragment detection, the lowest concentration measured was 4 nM (1.5 ng/µL) and the results revealed a high assay specificity for A. *sal* detection (Figure 1).

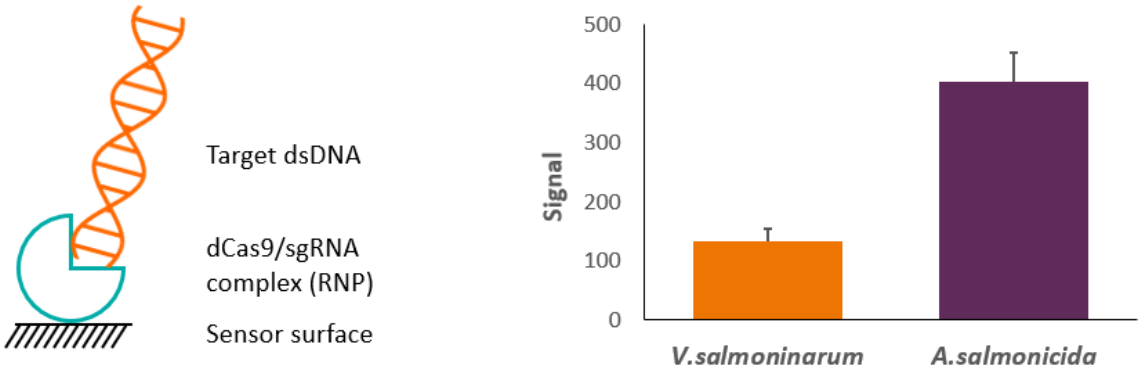

Figure 1. *Left: Schematic representation of the CRISPR/dCas9-chip based assay. Right: Specificity of CRISPR/dCas9 based assay using PCR fragments. Binding of 4 nM target dsDNA from A. sal and non-target dsDNA from V. sal. Each bar represents the average of values for three sensors ± SD.*

For the detection of whole genomic DNA, three different concentrations (30, 160, and 250 fM) of gDNA were measured. Figure 2 shows the signal output for both target and non-target DNA samples. The results indicate that the assay allows specific detection of 160 fM of target DNA. The lower detectable concentration of whole genomic DNA can be explained by the fact that it is much larger (4.8 Mbp for A. *Sal*, 3.1 Mbp for V. *Sal*) than the PCR fragments.

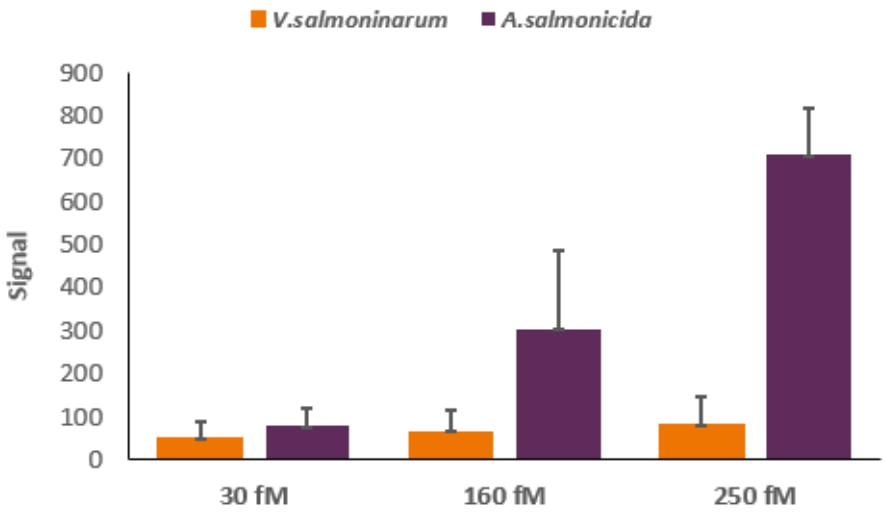

Figure 2. CRISPR/dCas9 based assay for whole genomic DNA detection. Whole genomic DNA from V. sal was used as a negative control, while whole genomic DNA from A. sal was used as target DNA. Each bar represents the average of values for three sensors ± SD.

Conclusion

The current work demonstrates the ability of Surfix's photonic diagnostics platform to detect bacterial target dsDNA using a novel CRISPR/dCas9 based assay which does not require cell culture or DNA amplification. This assay is simple, rapid, specific, and sensitive, with a detection level of 4 nM for PCR fragments and 160 fM for whole genomic DNA.

Surfix's objective is to advance the platform to enable easy-to-use, sensitive, and reliable detection of a wide array of molecular biomarkers, elevating pathogen detection and other diagnostic applications to the next level.

References

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[3] Huang, T. et al. CRISPR-Cas-based techniques for pathogen detection: Retrospect, recent advances, and future perspectives *J Adv Res* ***50***, 69-82 (2023). https://doi.org/10.1016/j.jare.2022.10.011

Abbreviations

aMZI	Asymmetric Mach-Zehnder Interferometer
A. *Sal*	Aeromonas *salmonicida*
bp	base pair
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
(d)Cas	(endonuclease deficient) CRISPR associated protein
(ss, ds, g)DNA	(single stranded, double stranded, genomic) Desoxyribonucleic acid
NGS	Next Generation Sequencing
PCR	Polymerase Chain Reaction
(sg)RNA	(single guide) Ribonucleic acid
RNP	Ribonucleoprotein
V. *Sal*	Vagococcus *salmoninarum*