A Label-free impedance biosensing assay based on CRISPR/Cas12a collateral activity for bacterial DNA detection

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The rapid and selective identification in the clinical setting of pathogenic bacteria causing healthcare associated infections (HAIs) is a major challenge, as the number of people affected worldwide and the associated mortality are on the rise. In fact, traditional laboratory techniques such culture and polymerase chain reaction (PCR)-based methodologies are often associated to high response times and are not able to response of this issue. Recently, a new class of programmable endonuclease enzymes called Cas proteins associated to clustered regularly interspaced short palindromic repeat loci (CRISPR) has revolutionized molecular diagnostics and biosensors field [1]. In this study, we present an electrochemical label-free biosensing assay based CRISPR/Cas12a to detect *Escherichia coli* (*E. coli*) and *Staphylococcus aureus (S. aureus)*. The programmable Cas12a endonuclease activity, induced by a specific guide RNA (gRNA), and the triggered collateral activity were assessed in in vitro restriction analyses (Figure1 a,b), and evaluated thanks to electrochemical impedance spectroscopy measurements using a modified electrode (Figure1c) [2]. The Cas12a/gRNA system was able to specifically recognize amplicons from different clinical isolates of *E. coli* and *S. aureus* with a limit of detection of 3nM and a response time approximately of 80 minutes.

References

[1] A. Bonini, N. Poma, F. Vivaldi, A. Kirchhain, P. Salvo, D. Bottai, A. Tavanti, F. Di Francesco, J. Pharm. Biomed. Anal. 2021, 192, 113645.
[2] A. Bonini, N. Poma, F. Vivaldi, D. Biagini, D. Bottai, A. Tavanti, F. Di Francesco, A Label-free impedance biosensing assay based on CRISPR/Cas12a collateral activity for bacterial DNA detection, J. Pharm. Biomed. Anal. 2021, (Under review).

Figures

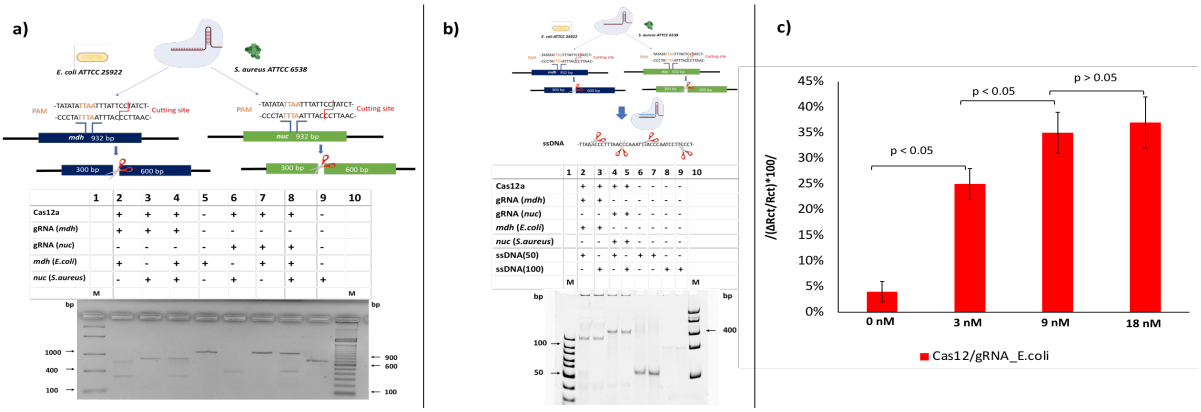

Figure 1. a) Visualization of the CRISPR/Cas12a cleavage specificity on a 1% agarose gel; b) 10% PAGE in TBE 1X. The gel shows Cas12a collateral activity upon cleavage of a ssDNA reporter; c) EIS Biosensing assay calibration response of Cas12/gRNA Vs E. coli target in the range from 3nM to 18nM .