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IN-VITRO TRANSCRIPTION-BASED BIOSENSORS: Is it Possible to Achieve Rapid and Low-Cost Detection of Lead in Real Samples?

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Approximately 10% of Argentina's population lives in the Matanza-Riachuelo Basin, where the various anthropogenic activities generated within the framework of the economic and social processes of the region, implicate a severe impact on the environment, specifically on the quality of the water. Currently, water quality analysis is carried out by expensive equipment, which also implies the need for transportation, equipped facilities and trained professionals. Synthetic biology has emerged as a promising strategy to develop economically accessible, easy-to-use, and transportable biosensors that can be locally produced facilitating environmental monitoring. Here, we present the optimization of a cell-free based platform to determine the presence of lead in environmental samples. Our cell-free *in vitro* transcription platform allows for the detection of lead among other heavy metals and small molecule contaminants in water samples. ROSALIND (RNA Output Sensor Activated by Ligand Induction), is a semi-quantitative biosensor, consistent of a highly processive RNA polymerase, bacterial transcription factors and synthetic DNA strand that transcribes a fluorescence-activating RNA aptamer. Previous efforts in the laboratory allowed local application of the biosensor and initial assays with water samples from the basin. Here we focus on evaluating and optimizing the reaction after lyophilization, as this process is key to ensuring on-site detection of this contaminant. Tuning of the freezer-dried process showed that the polymerase activity remained effective and the regulatory capacity of the transcription factor was preserved. This grants easy storage and distribution, facilitating on-site quality analysis of water or other complex matrices. As specificity is a key characteristic for the performance of biosensor, we then turned to assess its specificity toward lead. Water samples from the basin were tested to evaluate possible interferences. We observe that interference with zinc ions is problematic with some samples. Thus, we evaluate a strategy based on DNA circuits to solve crosstalk, taking advantage of transcription factors specific for these contaminants.

Palabras clave: biosensor - water contaminants - heavy metals - in vitro transcription