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# Potential of using a highly sensitive cell-free fluoride riboswitch-based biosensor for detecting water quality

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Ensuring water quality is critical for human health and essential for everyday household use, as excessive fluoride intake can lead to dental and skeletal fluorosis while insufficient fluoride increases the risk of dental decay, tooth sensitivity, and weakened skeletal health. The conventional methods for fluoride detection such as fluoride ion selective electrode and ion exchange chromatography suffer from drawbacks such as low sensitivity and unsuitability for on-site detection. Furthermore, these methods are only capable of detecting fluoride levels in the micromolar range, which creates a demand for a highly sensitive method capable of accurately detecting fluoride levels in water samples. Riboswitches are specific regions of the 5' untranslated region of prokaryotic mRNA that can bind specific ligands, such as fluoride ions, and thereby act as regulatory elements by undergoing conformational changes. As riboswitches are extremely selective, they indicate high sensitivity to low ligand levels. This study focused on the development of a novel cell-free biosensor from the fluoride responsive riboswitch (FRS) of *Bacillus cereus*, featuring a highly selective DNA-based FRS (aptamer) labelled with black hole quencher-1 (BHQ-1) and an expression platform labelled with fluorescein amidite (FAM) fluorophore for the detection of fluoride in aqueous samples through a fluorescence resonance energy transfer (FRET) assay. Optimum conditions for fluoride binding with the biosensor were determined using a denaturing urea PAGE, revealing an optimum $Mg^{2+}$: biosensor ratio of 2:1. Following this, the FRET assay was conducted using the Synergy HTX microplate reader to determine and optimise assay parameters, including aptamer concentration (1 μmol/L), incubation time (25 min), and sample volume (20 μL). The experimental results indicated a strong linear correlation between fluoride concentration and fluorescence intensity with a significant $R^2$ value of 1.000 at very low nanomolar levels (300–1000 nmol/L), showcasing the potential of this biosensor for detecting low nanomolar fluoride levels in aqueous samples, indicating a promising application for water quality assurance.

**Keywords:** Riboswitch biosensor, Nanomolar fluoride detection, FRET assay, Cell-free biosensor, Denaturing urea PAGE