Electrochemical Biosensor Utilizing CRISPR/Cas12a Amplification for the Detection of *E.coli*

Chenyan Li^{a,1}, Yilan Liang^{b,1}, Qincong Feng^{1,c,*}

^a The Second Affiliated Hospital of Guangxi Medical University, Department of

Laboratory, Liuzhou Cancer Hospital, of Guangxi, Guangxi 545000, China.

^b Guangxi Medical University, Guangxi, 530021, China

^c The 923rd Hospital of the Chinese People's Liberation Army Joint Logistics Support

Force, Guangxi, 530000, China

* Corresponding authors.

E-mail addresses: FQC923@163.com (QC. Feng)

 Table S1: The nucleic acid sequences used in this work.

	
note	sequence (5'-3')
Target	E.coli-2571
Apt A	CCA TGA GTG TTG TGA AAT GTT GGG ACA CTA GGT GGC ATA GAG CCG
Apt B	TGT CCC AAC ATT TCA CAA
DNA	SH-GCA TTT ATT GCA-Fc
PLP	TTG TGA TTG GTT TAG TTG TTT ATC TAC ACT TAG TAG AGT GTT
TS	ACA GGG TTG GTT TAG TTG TTT ATC TAC ACT TAG TAG AGT GTT
NTS	CTA CTA AGT GGG CCC GTA GGG CCC AAC CAA
crRNA	UAA UUU CUA CUA AGU GUA GAU AAA CAA CCA CTA AAC CAA

1.1 Stability testing

For electrochemical sensors to be used in real-world clinical settings, stability is essential. In this work, we assessed the CRISPR/Cas12a protein and nucleic acid sequences' capacity to withstand storage by storing them for fifteen days at four, twenty, and eighty degrees Celsius. The electrochemical sensors were cooled to room temperature for every measurement by means of a buffer solution. To evaluate the stability of the storage, we tracked the electrochemical current intensity every day. Notably, the biosensor retained a minimum of 89% of its original current response, suggesting that it is stable enough to identify *E.coli*. Furthermore, Figure 1 offers additional proof of the great stability of CRISPR/Cas12a sequences and proteins under various storage scenarios.



Figure S1: SWV (Square wave voltammetry) for storage stability. (A) the first day; (B) the fifteenth day;; a) -80 $^{\circ}$ C; b) -20 $^{\circ}$ C; c) 4 $^{\circ}$ C.

1.2 Description of testing environment

The water samples used in the testing were lake filtered water, which was designed to meet the living environment of *E. coli-2571* as well as the testing needs. The water samples used in the recovery experiments were pure water and pure milk.



Figure S2. (A) Photo of a lake. (B) Top view of water sample a) lake water; b) preliminary filtered lake water; c) pure water. C) Front view of water sample a) lake water; b) Preliminary filtered lake water; c) pure water.