

Electronic Supplementary Information:

A PCR-Free Screen-Printed Magnetic Electrode for the Detection of Circular RNA from Hepatocellular Cancer Based on Back-Splice Junction

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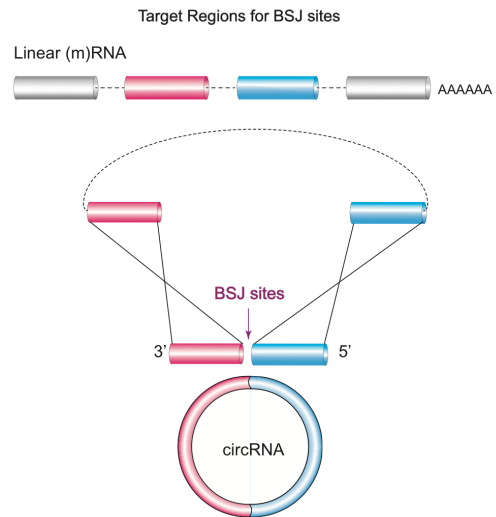


Fig. S1. Schematic illustration of Back-splice junction (BSJ) sites of circRNA. CircRNAs are produced by precursor mRNA back-splicing of exons of genes. BSJ sites of circRNA are considered to be a conserved motif.

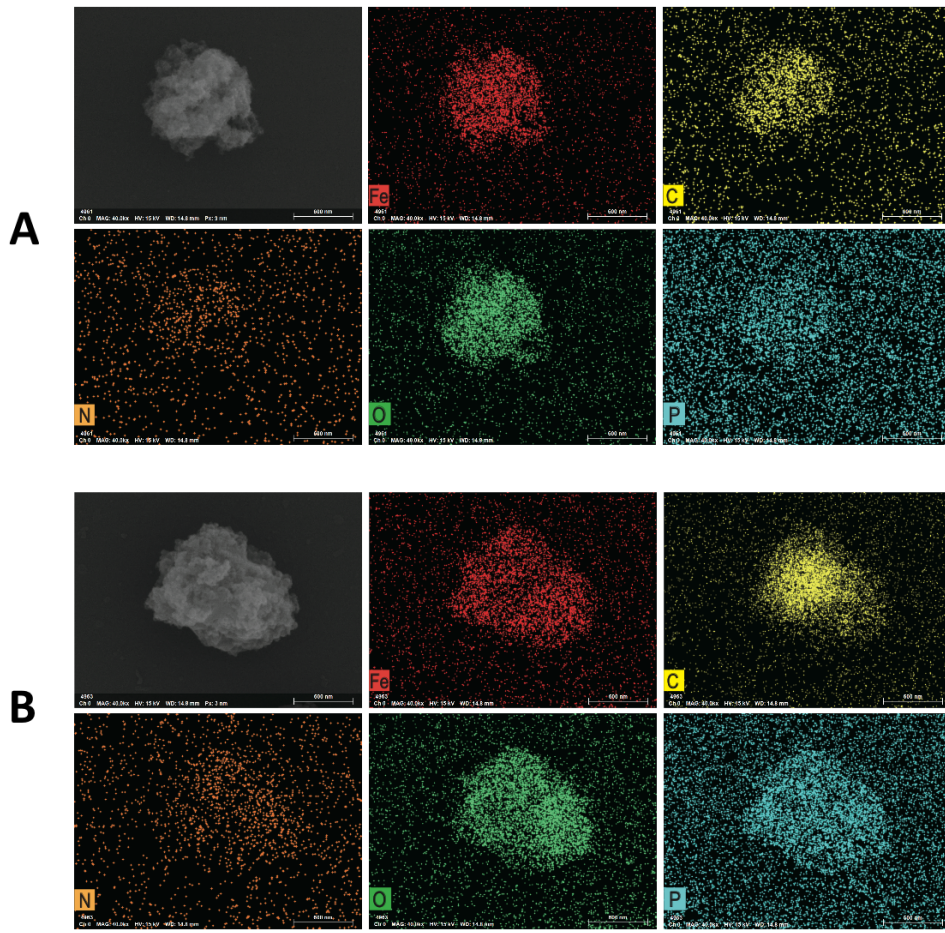


Fig. S2. EDS map of (A) streptavidin-coated magnetic beads. (B) biotin-probes conjunct streptavidin-coated magnetic beads.

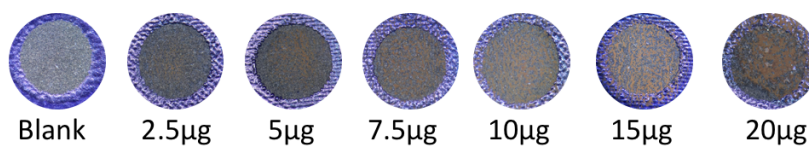
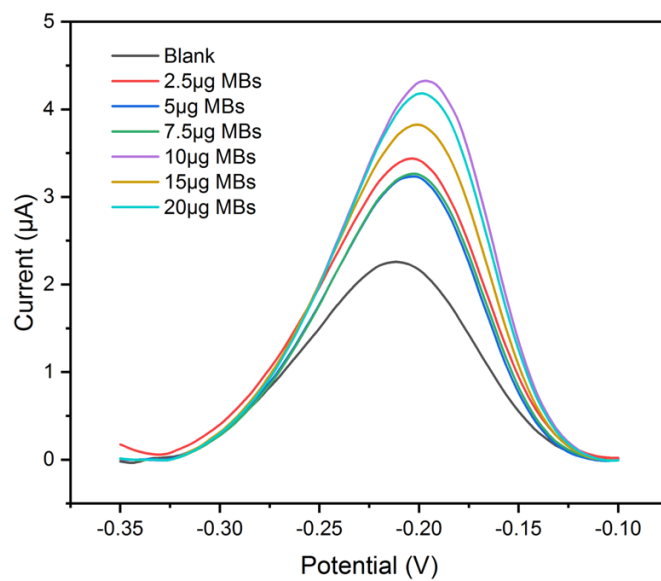


Fig. S3. Various amounts of suspension volume ranging from 0 to 20 μg MBs in electrochemical performance AND surface of the electrode.

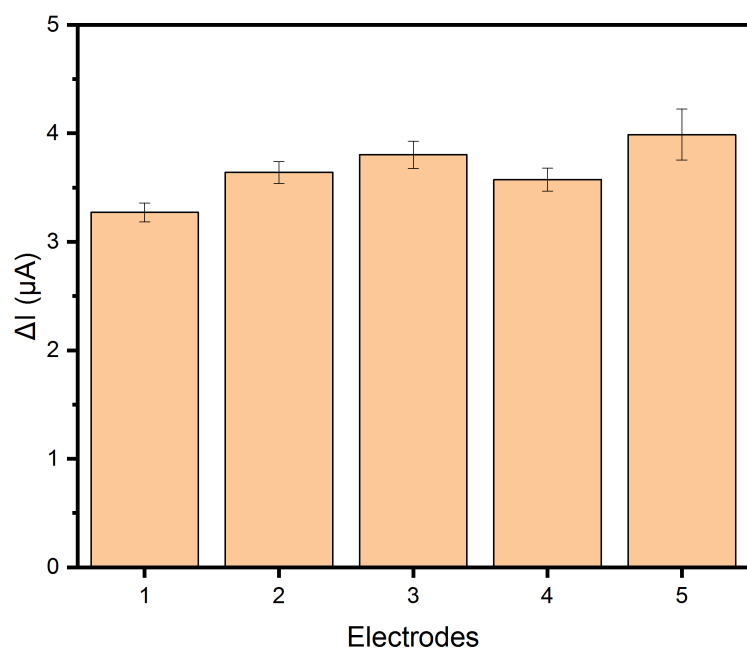


Fig. S4. The electrochemical DPV response of independently fabricated 5 biosensors in 10pM concentrations. Error bars represented the results of three measurements in the same electrode.

Table S1. Oligonucleotides used in this work

Oligonucleotide	Sequence(5'→ 3')
BSJ sequence of circCDYL (20bp)	5'-UAACGGGAAAGGUUGAAAGG-3'
Captured probe of BSJ-circCDYL	5'-biotin-CCTTTCAACCTTTCCGTTA-3'
circCDYL Synth	5'-CUGUUAACGGGAAAGGUUGAAAGGAUUG-3'
3-base mutation circCDYL Synth	5'-CUGUUAACGGUAA CGG GUGAAAGGAUUG-3'
microRNA21	5'-UAGCUUAUCAGACUGAUGUUGA-3'

Table S2. Comparison of different methods for circRNA analysis.

Method	Electrode	Technique	Reverse Transcription	Any Nuclease Needed	RNase R Needed	Type of Probe	Limit of Detection	Linear Range	Time	Ref
Northern blot			Yes	Yes	Yes			~10 nM	>4H	¹
Real-time quantitative PCR			Yes	Yes	Yes		100fM		>4H	²
Electrochemical	Gold disk electrodes	SWV	No	Yes, DSN	No	BSJ-DNA	3.47fM	10 fM-100 pM	About 2H	³
Electrochemical	SPME	DPV	No	No	No	BSJ-DNA	1pM	1 pM-1 nM	Less 3H	this work

PCR: Polymerase Chain Reaction; SPME: Screen-Printed Magnetic Electrode; SWV: Square Wave Voltammetry; DPV: Differential Pulse Voltammetry; DSN: Duplex-Specific Nuclease; BSJ-DNA: Back-Splice Junction-DNA.

Table S3 Determination of circRNA added in human serum

Serum sample	Add/pM	Found/pM	Recovery/%	RSD/% (n=3)
1	1	1.197	119.7	8.34
2	10	11.51	115.1	8.94

Reference:

1. A. D. Castaneda, N. J. Brenes, A. Kondajji and R. M. Crooks, *J Am Chem Soc*, 2017, **139**, 7657-7664.
2. B. C. Yin, Y. Q. Liu and B. C. Ye, *J Am Chem Soc*, 2012, **134**, 5064-5067.
3. J. Jiao, C. Li, L. Ning, L. Shi, L. Wang, Y. Xiang and G. Li, *Sensors and Actuators B: Chemical*, 2020, **302**.