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*Corresponding author: Zhang J, Beijing chaoyang, TianShuiYuan street no. 6, Beijing Inspection And Quarantine Testing Center, Beijing Entry-Exit Inspection Quarantine Bureau, Beijing, China, 100026, Tel/Fax: +86-10-58619231; E-mail:

National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention; Biotin-AC5-Sulfo-Osu was purchased from Dojindo Laboratories (Kumamoto, Japan). Streptavidin and adenosine diphosphate (ADP) were purchased from Sigma (St. Louis, MO, USA). N-(uorescein-5-thiocarbamoyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine,

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electrophoresis and hybridization; Due to large reduction of these usual operations the molecular motor biosensor greatly reduced the likelihood of contamination [28]. Molecular motor biosensor has rapid and precise properties and if we can make it subminiature and portable, or make it directly to detect at the scene of sample, it could be wildly applied. Predictably, along with the progress of micromachining technology and nanotechnology [29-32], the molecular motor biosensor will be largely applied in the various detection fields.

Establishment of the molecular motor biosensor reaction system

Firstly, ChroRV and ChroHAV were diluted by concentration gradient. In the different concentration conditions, the fluorescence value of H_2O and virus was detected, and the fluorescence differentials were shown as Figure 1. From the results, we found that the dilution radio is lower and the fluorescence intensity of final reaction system is higher. Because along with the concentration of molecular motor biosensor increasing, the reaction system has more ATPase, and therefore fluorescence intensity is larger. Based on the principle of maximum differentials between molecular motor and H_2O, we finally chose the 0.015 mg/mL, 0.0173 mg/mL and 0.0260 mg/mL as the final concentration of ChroRV and ChroHAV respectively. Meanwhile, we have explored the optimum reaction concentrations of all three virus RNA and data was shown as Supplemental Figure S2. We found that the fluorescence values of different concentration of RNA were all higher than the control (H_2O), especially 0.6 ng/ L and 0.9 ng/ L of RV and HAV respectively indicating that the detection of F_0F_1-ATPase molecular motor biosensor is effective and the optimum concentration of RNA is 0.6 ng/ L and 0.9 ng/ L of RV and HAV respectively.

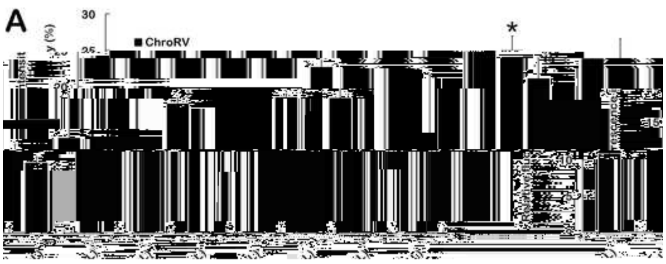

Figure 1: Fluorescence values of H 2 2 DQG GLIIHUHQW FRQFHQWUDWLRQ RI 51\$ RI 59 DQG +\$9 \$ 7KH GHWHFWLRQ RI 59 51\$ XQGHU GHWHFWLRQ RI +\$9 51\$ XQGHU PJ P/ &KUR+\$9 as mean values ±SD of three separate experiments. The bars represent SD of PHDQ YDOXHV DQG WKH DVWHULVNV LQGLFDWH VWDWLVWLFDO VLJQL¿FDQFH (p<0.05).

In order to detect whether there were cross reactions of F_0F_1-ATPase molecular motor biosensor, we furthermore tested the speci city of ChroRV and ChroHAV respectively in RV, HAV and norovirus (NV) detection and data was shown as Figure 2. In RV detection, the molecular-biosensor uorescence intensity of RV was signi cantly higher than the uorescence intensity of H2O, however, the uorescence intensity of HAV and NV was basically unchanged compared with the control indicating that the molecular motor biosensor of ChroRV is speci c RNA and has none cross reactions

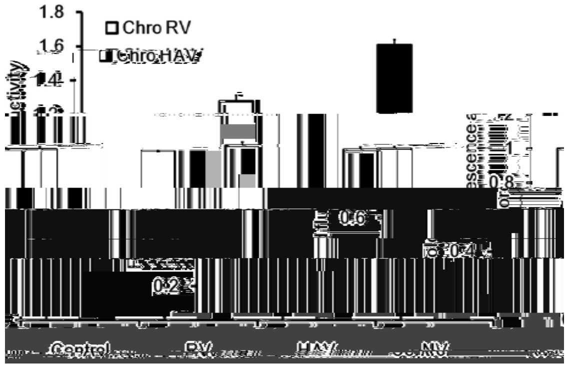

Figure 2: Cross reaction analysis between ChroRV and ChroHAV respectively LQ 59 +\$9 DQG 19 GHWHFWLRQ \$ &KUR59 GHWHFWLRQ % &KUR+\$9 GHWHFWLRQ)OXRUHVFHQFH YDOXH GLIIHUHQFH FRPSDUHG ZLWK + 2 (p<0.05).

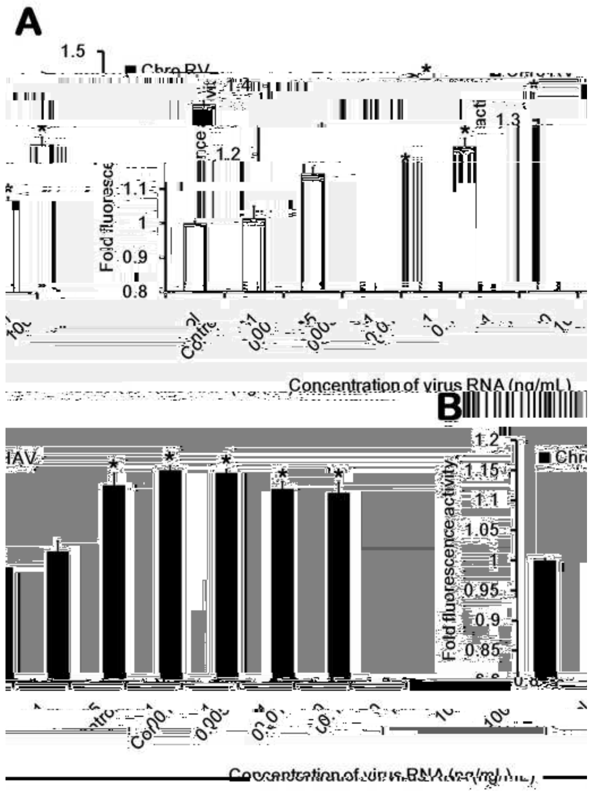

Figure 3: The sensitivity analysis of ChroRV and ChroHAV detection respectively under different RV and HAV concentration. (A) Fluorescence intensity of PJ P/ &KUR59 % 7KH &KUR+\$9 XQGHU VHULRXV FRQFHQWUDWLRQ RI 59 51)OXRUHVFHQFH YDOXHV RI VDPSOHV DUH VLJQL¿FDQWO\ KLJKHU WKDQ + 2 (p<0.05).

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reovirus and rotavirus infections. Avian Dis 36: 183-188.

10. Saif LJ, Bohl EH, Kohler EM, Hughes JH (1977) Immune electron microscopy of transmissible gastroenteritis virus and rotavirus (reovirus-like agent) of swine. Am J Vet Res 38: 13-20.

11. 6XJLKDUD 5 5HXSNH + 6FKPLGW :HVWKDXVHQ $ 3RKOH + *HOGHUEORP +5 HW DO 1HJDWLYH VWDLQLQJ (0 IRU WKH GHWHFWLRQ RI (SVWHLQ %DUU YLUXV LQ RUDO KDLU\ OHXNRSODNLD - 2UDO 3DWKRO 0HG

12. &XL = =KDQJ) <XH - 'HWHFWLQJ SURWRQ ÀX[DFURVV FKURPDWRSKRUHV GULYHQ E\ )) $73DVH XVLQJ 1 ÀXRUHVFHLQ WKLRFDUEDPR\O dihexadecanoyl-sn-glycero-3-phosphoethanolami ne, triethylammonium salt. Anal Biochem 344: 102-107.

13. 6KX * <XH -& 2X <DQJ F_0F_1-ATPase, rotary motor and biosensor. 1DQRVFDOH

14. Fillingame RH (1997) Coupling H+ transport and ATP synthesis in F1F0-ATP synthases: glimpses of interacting parts in a dynamic molecular machine. J Exp Biol 200: 217-224.

15. 6WRFN ' /HVOLH $* :DONHU -(0ROHFXODU DUFKLWHFWXUH RI WKH URWDU\ PRWRU in ATP synthase. Science 286: 1700-1705.

16. <DVXGD 5 1RML + <RVKLGD 0 .LQRVLWD .- ,WRK + 5HVROXWLRQ RI GLVWLQFW URWDWLRQDO VXEVWHSV E\ VXEPLOOLVHFRQG NLQHWLF DQDO\VLV RI) $73DVH 1DWXUH 410: 898-904.

17. *DR <4 <DQJ : .DUSOXV 0 $ VWUXFWXUH EDVHG PRGHO IRU WKH V\QWKHVLV and hydrolysis of ATP by F_1-ATPase. Cell 123: 195-205.

18. 6DEEHUW ' (QJHOEUHFKW 6 -XQJH : ,QWHUVXEXQLW URWDWLRQ LQ DFWLYH) $73DVH 1DWXUH

19. (OVWRQ 7 :DQJ + 2VWHU * (QHUJ\ WUDQVGXFWLRQ LQ $73 V\QWKDVH 1DWXUH

20. .LQRVLWD .- <DVXGD 5 1RML + $GDFKL . $ URWDU\ PROHFXODU PRWRU WKDW FDQ ZRUN DW QHDU HI¿FLHQF\ 3KLORV 7UDQV 5 6RF /RQG % %LRO 6FL 473-489.

21. 1RML + <DVXGD 5 <RVKLGD 0 .LQRVLWD .- 'LUHFW REVHUYDWLRQ RI WKH rotation of F_1 $73DVH 1DWXUH

22. <DVXGD 5 1RML + .LQRVLWD .- <RVKLGD 0 F_1-ATPase is a highly efficient molecular motor that rotates with discrete 120 degree steps. Cell 93: 1117-1124.

23. =KDQJ :DQJ ; ;X 0 =KDQJ + =KDQJ / HW DO F_0F_1-ATPase Molecular Motor Biosensor for Rapid Detection of Hepatitis A Virus in Foods. Food Science: 178-182.

24. =KDQJ ;X 0 :DQJ ; :DQJ < :DQJ ; HW DO 'HWHFWLRQ RI ERUQH URWDYLUXV E\ PROHFXODU PRWRU ELRVHQVRU Bao 29: 681-690.

25. 6X]XNL 7 0XUDNDPL 7 ,LQR 5 6X]XNL - 2QR 6 HW DO F_0F_1-ATPase synthase is geared to the synthesis mode by conformational rearrangement of epsilon subunit in response to proton motive force and ADP/ATP balance. J Biol Chem 278: 46840-46846.

26. Cheng J, Zhang XA, Shu YG, Yue JC (2010) F_0F_1-ATPase activity regulated by external links on beta subunits. Biochem Biophys Res Commun 391: 182-186.

27. =KDR :DQJ 3 :DQJ) =KRX + /L : HW DO regulated by the rotator of F_0F_1-ATPase to detect deoxynivalenol rapidly. Biochem Biophys Res Commun 423: 195-199.

28. =KDQJ /L = =KDQJ + :DQJ - /LX < HW DO 5DSLG GHWHFWLRQ RI foodborne pathogens by F0F1-ATPase molecular motor biosensor. J Microbiol Methods 93: 37-41.

29. $WDU 1 (UHQ 7 <ROD 0/ :DQJ 6 $ VHQVLWLYH surface plasmon resonance nanosensor for selective determination of trace triclosan in wastewater. Sensors and Actuators B: Chemical 216: 638-644.

30. 0DOHK -DYD]PL)7 $WDU 1 <ROD 0/ *XSWD 9. HW '1$ %LRVHQVRU %DVHG RQ D 3HQFLO *UDSKLWH 3RO\SUROH)XQFWLRQDOL]HG 0XOWLZDOOHG &DU 6-Mercaptopurine Anticancer Drug. Ind Eng Chem Res 54: 3634-3639.

31. $WDU 1 (UHQ 7 <ROD 0/ :DQJ 6 $ PROHFXODU LPSULQ sensitive determination of citrinin in red yeast rice. Food Chem 184: 7-11.

32. $WDU 1 (UHQ 7 <ROD 0/ 0DOHK +. 'HPLUGRJHQ % and iron oxide@gold nanoparticle anchored nitrogen and sulfur-functionalized reduced graphene oxide electrocatalyst for methanol oxidation. RSC Advances 5: 26402-26409.