

# Rolling Circle Amplification-Enabled Ultrasensitive Point-of-Care Test Method for Aflatoxin B1 in the Environment and Food

Hongyu Duan <sup>1,†</sup>, Yuan Zhao <sup>1,†</sup>, Xiaofeng Hu <sup>1</sup>, Meijuan Liang <sup>1</sup>, Xianglong Yang <sup>1</sup>, Li Yu <sup>1</sup>, Behrouz Tajdar Oranj <sup>2</sup>, Valentin Romanovski <sup>3</sup>, Peiwu Li <sup>1</sup> and Zhaowei Zhang <sup>1,\*</sup>

<sup>1</sup> Oil Crops Research Institute of Chinese Academy of Agricultural Sciences, Wuhan 430062, China; dhy2634591222@163.com (H.D.); zhao\_yuan2494@126.com (Y.Z.); xiaofenghu85@126.com (X.H.); liangmj@whu.edu.cn (M.L.); xianglongyang@foxmail.com (X.Y.); yuli01@caas.cn (L.Y.); peiwuli@oilcrops.cn (P.L.)

<sup>2</sup> Research Center for Environmental Determinants of Health (RCEDH), Kermanshah University of Medical Sciences, Kermanshah 67146, Iran; tajdar.tums@yahoo.com

<sup>3</sup> Center of Functional Nano-Ceramics, National University of Science and Technology MISIS, Moscow 101000, Russia; vramano@kth.se

\* Correspondence: zwzhang@whu.edu.cn

† These authors contributed equally to this work.

## S1. In vitro validation methods

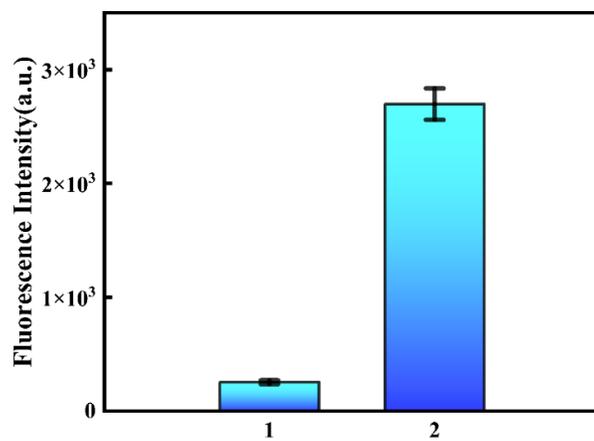
**Validation of RCA reaction system in tubes:** the RCA reaction system contained 1  $\mu\text{M}$  circular DNA, 1 mM dNTP, 0.5 units/ $\mu\text{L}$  phi29 DNA polymerase, 0.2 mg/mL BSA and 1  $\times$  phi29 buffer. First, 20  $\mu\text{L}$  of the RCA reaction system was added to the final concentration of 1  $\mu\text{M}$  biotinylated primer in a PCR tube at 37  $^{\circ}\text{C}$  for 0, 1, 2, and 3 h, respectively. After the RCA reaction, the tubes were incubated at 65  $^{\circ}\text{C}$  for 10 min. The RCA products were characterized by 12% native PAGE analysis. After electrophoresis analysis for 50 min at 120 V, the gel was submerged for 25 minutes in a solution containing GelRed dye to stain DNA lands and visualized under 302 nm UV light.

**Verification of the RCA reaction in microwells:** 10  $\mu\text{g/mL}$  of streptavidin was preloaded on microwells. Meanwhile, after streptavidin was preloaded on microwells, we conducted the RCA reaction in microwells following the process described below. 50  $\mu\text{L}$  of the RCA reaction system was added into a microwell at 37  $^{\circ}\text{C}$  for 3 h. After washing the microwells with PBST, 50  $\mu\text{L}$  of the FAM-labelled signal probes (1  $\mu\text{M}$ ) were added to combine with RCA products and incubated at 37  $^{\circ}\text{C}$  for 1 h. Finally, after washing the microwells with PBST, the fluorescence intensity was recorded by using a SpectraMax i3x Microplate Reader at the excitation wavelength (498 nm) and emission wavelength (512 nm).

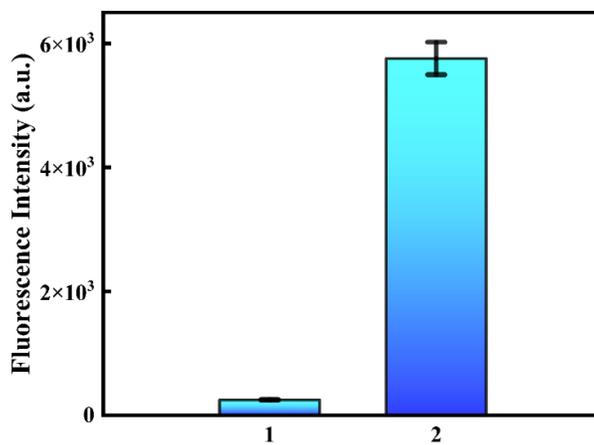
## S2. HPLC parameters

The HPLC was K2025 (Wooking, China) equipped with photochemical derivation, and the chromatographic column was Agilent TC-C18 (4.6 mm  $\times$  150 mm, 5  $\mu\text{m}$ ). The mobile phase was 45% methanol, the running time was 12 min, the flow rate was 0.8 mL/min, the injection volume was 10  $\mu\text{L}$ , and the column temperature was 35  $^{\circ}\text{C}$ . The excitation and emission wavelengths were 360 nm and 440 nm, respectively.

### S3. Figures



**Figure S1.** Feasibility of the RCA reaction with streptavidin preloaded on microwells. (1) Without biotinylated primer, (2) with biotinylated primer. (n=3)



**Figure S2.** Feasibility of the RCA-SMIP on microwells. (1) Without biotinylated mAb, (2) with biotinylated mAb. (n=3)

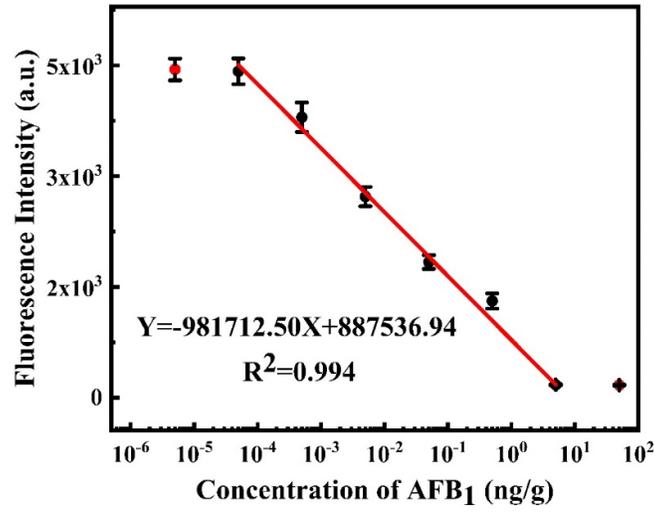


Figure S3. The corresponding linear calibration plots for AFB1 detection in peanut soil samples.

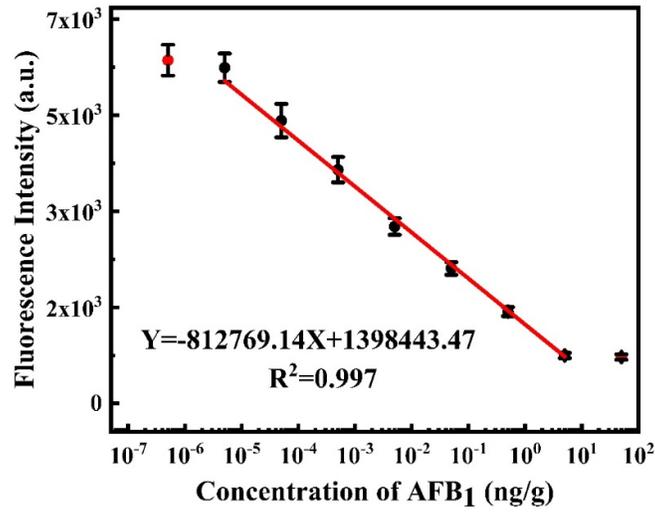


Figure S4. The corresponding linear calibration plots for AFB1 detection in irrigation water samples.