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# A novel fluorescent biosensor for detection of target DNA fragment from the transgene cauliflower mosaic virus 35S promoter

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ABSTRACT

In this paper, we reported a convenient fluorescence method for the detection of genetically modified organisms (GMOs). As it is known that the cauliflower mosaic virus (CaMV) 35S promoter is widely used in most transgenic plants (Schnurr and Guerra, 2000), we thus design a simple method based on the detection of a section target DNA (DNA-T) from the transgene CaMV 35S promoter. In this method, the full-length guanine-rich single-strand sequences were split into fragments (Probe 1 and 2) and each part of the fragment possesses two GGG repeats. In the presence of $K^+$ ion and berberine, if a complementary target DNA of the CaMV 35S promoter was introduced to hybridize with Probe 1 and 2, a G-quadruplex–berberine complex was thus formed and generated a strong fluorescence signal. The generation of fluorescence signal indicates the presence of CaMV 35S promoter. This method is able to identify and quantify Genetically Modified Organisms (GMOs), and it shows wide linear ranges from $5.0\times10^{-9}$ to $9.0\times10^{-7}$ mol/L with a detection limit of $2.0\times10^{-9}$ mol/L.



## 1. Introduction

Nowadays, the use of genetically modified organisms (GMOs) is becoming more and more widespread in agriculture and food industry (Levandi et al., 2008). The GMOs have some advantages, such as improving the resistance of crops and providing better nutritional properties (Koziel et al.,1993; Gao et al., 2000). However, it also raises social and ethical concerns because of its environmental risk and biosafety (Huang and Pan, 2004). Therefore, many countries are subject to compulsory labeling (Regulation (EC) 258/97, Council Regulation (EC) 1139/98) for the food and feed consisting GMOs in market, so it is very interesting to develop the rapid methods for detection of GMOs. Several methods have been reported for the quantitative analysis of GMOs, such as enzyme-linked immunosorbent assay (ELISA) (Petit et al., 2003; Monaghan et al., 2008), polymerase chain reaction (PCR) (Xu et al., 2006; Brodmann et al., 2002; Huang and Pan, 2004), microarray (Germini et al., 2004) and electrochemistry (Ahmed et al., 2009; Zhu et al., 2008a,2008b). Usually, polymerase chain reaction (PCR) methods are preferred for GMOs detection for its high sensitivity and stability (García-Cañas et al., 2002; Sun et al., 2008), but it has some unavoidable drawbacks such as complicated and labor-intensive procedures, expensive running cost and time-consuming (Zhu et al., 2008a,2008b; Chaouachi et al., 2008). Therefore, new analytical methods which can handle simple and rapid detection of GMOs are urgently needed. Herein, we developed a novel fluorescent sensor for rapid detection of the target DNA of GMOs based on the formation of berberine-G-quadruplex complex.

G-quadruplexes derived from G-rich sequences are four-stranded structures (Li et al., 2009). It has been reported that the unique structure of the four GGG made it possible to split the whole sequence into two parts (Deng et al., 2008). At the same time, such method leads to an extraordinary selectivity of nucleic acid recognition (Kolpashchikov, 2006). Recently, many examples of such split strategy have been reported. For instance, Deng used the split G-quadruplex as a sensitive probe for the identification of single nucleotide polymorphisms by giving a color signal (Deng et al., 2008). Kolpashchikov developed a split DNA enzyme for visual single nucleotide polymorphism typing (SNP) with 3-3′-diaminobenzidine tetrahydrochloride (DAB) (Kolpashchikov, 2008). In this paper, we used a split mode to develop a new DNA probe GMOs detection.

Berberine is well known of an active constituent of Chinese herbs. Also, it is able to induce and stabilize the formation of G-quadruplex (Zhang et al., 2007). Berberine is found to have a higher binding selectivity for the G-quadruplex DNA than duplex DNAs (Franceschin et al., 2006). In addition, the binding of

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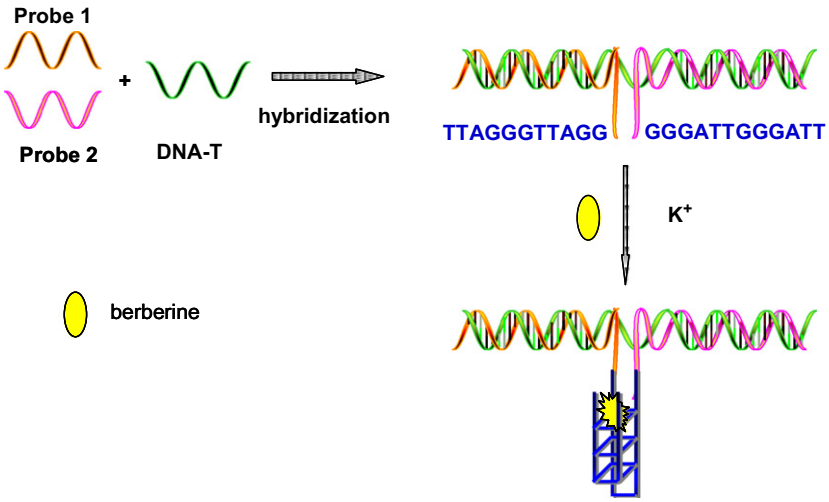


**Scheme 1.** Structure and sensing mechanism of GMOs for the designed sensor .

berberine to G-quadruplex generates a strong fluorescence signal (Arora et al., 2008).

A convenient fluorescence method can be introduced for the detection of GMOs based on the selectivite binding of berberine with G-quadruplex DNA. We know that CaMV 35S promoter exhibits a high level of transcriptional activity in various kinds of plant tissues and it is one of the most widely used promoters in transgenic plant research (Schnurr and Guerra, 2000). In our study, we utilized a 2:2 split mode to divide the unabridged guanine-rich single-strand sequences into two parts (Probe 1 and 2) and each part possesses two GGG repeats. As a complementary DNA of the target CaMV 35S promoter was introduced into each probe, they can hybridize with the target DNA fragment.

Scheme 1 illustrates the structure and the sensing mechanism of GMOs sensor. Without an appropriate cation, probe 1 and probe 2 only exist mainly in the dissociated form. When adding a target DNA-T, one side of probe 1 and probe 2 would hybridize with it under appropriate conditions. Simultaneously, the other side of probe 1 and probe 2 would also be fixed and get close to each other. In the presence of $K^+$ and berberine, a G-quadruplex–berberine complex was formed and generated a strong fluorescence signal. The fluorescence intensity was increased with the increasing of the concentration of the DNA-T. Based on the fluorescence signal intenty, we can quantify the concentration of the DNA-T.

## 2. Experimental

### 2.1. Apparatus and reagents

Most of GMOs contain cauliflower mosaic virus (CaMV) 35S promoter. This promoter gene was selected as a DNA target sequence and the lectin were selected as a reference gene in this study. Moreover, DNA-T (ATTGTGCGTCATCCCT TACGTCAGTGGAG) and DNA-R (GAGGATGGATTT AAACCAGTCAGCACCG) were screened from the CaMV 35S promoter and lectin gene, respectively with Primer Premier 5 software. Binary probes that include CTCCACTGACGTAA TTAGGGTTAGGG (probe-1) and TTAGGGTTAGGGGGATGACGCACAAT (probe-2) for fluorimetric analysis of DNA-T were prepared. Both probes were designed to have two specific portions as shown in Scheme 1. One portion is for hybridizing with DNA-T, and another portion is for hybridizing with two GGG repeats. In the presence of DNA-T, the probes boost up G-quadruplex formation and the fluorescence intensity is then enhanced remarkably.

### 2.2. Instrumentation and reagents

All DNA sequences were synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China). They were dissolved in a Tris–HCl buffer as DNA stock solutions (50 μM, pH 7.5). The DNA stock solutions were stored at 4 °C. Berberine was purchased from Shaanxi Sciphar Biotechnology Co., Ltd. (Shaanxi, China). The stock solutions were in $4.3\times10^{-4}$ mol/L.

### 2.3. Fluorescence spectroscopic analysis

The fluorescence emission spectra of berberine in Tris–HCl buffer were collected from 510 nm to 650 nm by using a spectrofluorimeter (Varian, Eclipse) at room temperature. The excitation wavelength was set at 362 nm. For the sensor optimization, $(F_T-F_R)/F_R$ ($\triangle F=F_T-F_R$) was described as sensor signal, where $F_T$ is the fluorescence intensity of the sensor solution with addition of $5\times10^{-7}$ mol/L DNA-T; $F_R$ is the fluorescence intensity of the sensor solution with addition of $5\times10^{-7}$ mol/L DNA-R. For the sensor calibration curve acquisition, $F$ was plotted as sensor signal, where $F$ is the fluorescence intensity of the sensor solution with addition of different concentration of DNA-T. Analyses were always measured in triplicate, and the standard deviation was plotted as the error bar.

### 2.4. Experimental procedure

To prepare the sample solutions, 2.5 μL of each probe (probe 1 and probe 2, at $5.0\times10^{-5}$ mol/L) and different amounts of DNA-T were well-mixed. The resulting solution was incubated at room temperature. After 20 min, 50 mM Tris–HCl solution containing 100 mM KCl was added to make the final volume of 250 μL. Afterward, 6.0 μL berberine (at $4.3\times10^{-4}$ mol/L) was added to the mixture and incubated for another 50 min at room temperature. The fluorescent signals of the mixture were recorded with fluorescence spectrophotometer at room temperature. The working principle of this GMO sensor is shown in Scheme 1.

## 3. Results and discussion

### 3.1. Choice of the G-quadruplex

Fig. 1 shows the fluorescence spectra of free berberine and its complex with different DNAs. In this study, eleven different DNAs

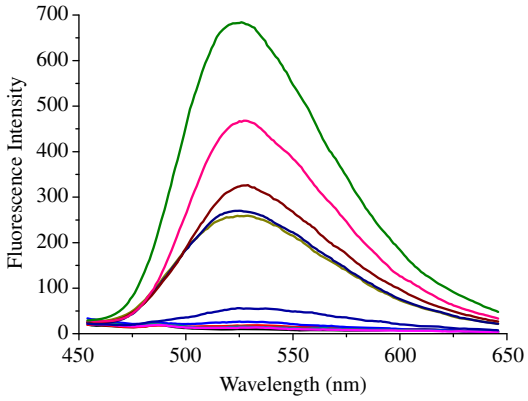


**Fig. 1.** Fluorescence spectra of berberine in the presence of different G-quadruplex DNAs 0.05 mol/L Tris–HCl (pH=7.5) containing 0.1 mol/L KCl with different G-quadruplex DNAs ($5\times10^{-7}$ mol/L) and berberine ($1.0\times10^{-5}$ mol/.L). The orders (top to bottom) of the DNAs are G-quadruplex DNAs: Hum24, Apt, Hum12, Tet12, Oxy 28, Oxy12; duplex DNAs: AT, GC, and LD; single-stranded DNAs: ssDNA1 and ssDNA2.

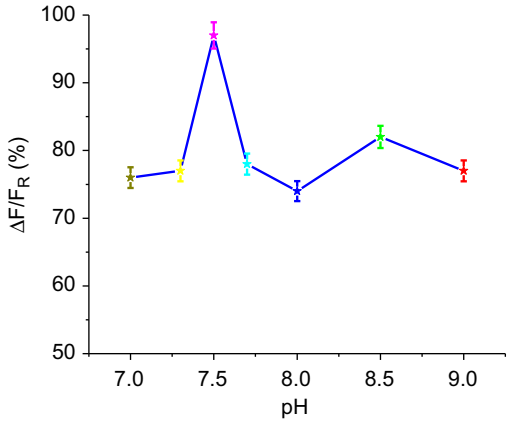


**Fig. 2.** Effect of buffer pH on $\triangle F/F_R$ 0.05 mol/L Tris–HCl containing 0.1 mol/L KCl with probes, DNA-T, DNA-R ($5.0\times10^{-7}$ mol/L) and berberine ($1\times10^{-5}$ mol/L), and the pH is 7.0, 7.3, 7.5, 7.7, 8.0, 8.5, 9.0, respectively.

**Table 1**
Oligonucleotides used in this work.

| Entry | Abbreviation | Sequence | Structure |
|---|---|---|---|
| 1 | ssDNA1 | 5′GAGCTCTCGAAAGAGCTCCGATTA | Single stranded |
| 2 | ssDNA2 | 5′TAGAGCACAGCCTGTCCGTG | Single stranded |
| 3 | LD | 5′GCGCA$_2$T$_2$GCGC | duplex |
| 4 | GC | 5′(GC)$_6$ | duplex |
| 5 | AT | 5′(AT)$_6$ | duplex |
| 6 | Tet12 | 5′(T$_2$G$_4$)$_2$ | G-quadruplex |
| 7 | Oxy12 | 5′G$_4$T$_4$G$_4$ | G-quadruplex |
| 8 | Hum12 | 5′(T$_2$AG$_3$)$_2$ | G-quadruplex |
| 9 | Apt | 5′G$_2$T$_2$G$_2$TGTG$_2$T$_2$G$_2$ | G-quadruplex |
| 10 | Oxy28 | 5′G$_4$(T$_4$G$_4$)$_3$ | G-quadruplex |
| 11 | Hum24 | 5′(T$_2$AG$_3$)$_4$ | G-quadruplex |

(see Table 1, entries 1–11) were used. From Fig. 1, it can be found that, at the same DNA base concentrations, the fluorescence intensities of berberine in the presence of G-quadruplex DNAs were always higher than those in the presence of duplex or single-stranded DNAs. In addition, the fluorescence enhancement in the presence of Hum 24 was highest among other G-quadruplexes, which indicates that berberine could be used as a sensitive biosensor, particularly for the quadruplex of Hum 24. To construct a binary probe, the sequence of Hum 24 was split into two parts (probe 1 and probe 2) and each part possesses two GGG repeats.

### 3.2. Optimization of sensing conditions

#### 3.2.1. Effect of pH

DNA is usually stable in neutral buffers. Therefore, using neutral buffer for the hybridization process is a suitable condition. In addition, berberine is a quaternary ammonium salt, and its fluorescence emission intensity is not related to pH conditions. However, it was reported that the pH changes would affect the formation of berberine-G-quadruplex complex (Liu et al., 2011). So, the effect of pH on $\triangle F/F_R$ is also investigated in our study. The experimental results taken place in the pH range of 7.0–9.0 were shown in Fig. 2. The results showed that $\triangle F/F_R$ were highest in pH 7.5, so pH 7.5 was chosen as the optimum condition for subsequent studies.

#### 3.2.2. Effect of incubation time between probes and DNA-T

We expected that $\triangle F/F_R$ is affected by the incubation time between probes and DNA-T. The results indicated that by increasing the incubation time, the enhancement rate was found increased accordingly. When the incubation time was over 20 min, the $\triangle F/F_R$ was gradually decreased. This was because in DNA-R system, more G-quadruplexes were formed with the increasing of incubation time, inducing the background fluorescence intensity increased. In this work, the suitable incubation time for the probes and DNA-T is 20 min.

#### 3.2.3. Effect of incubation time between G-quadruplex and berberine

To accelerate the detection of DNA-T using berberine as fluorescent indicator, the effect of incubation time between G-quadruplex and berberine was also investigated. The experiment results showed that a highest $\triangle F/F_R$ value was obtained as the incubation time is 50 min. With respect to sensitivity and speed, the incubation of 50 min was chosen for subsequent study.

### 3.3. Identification of DNA-T

Under the optimal conditions, we investigated the specificity of this detection system toward target sequence DNA-T relative to DNA-R (an endogenous gene for soybean). The addition of DNA-R shows no significant effect on the fluorescence intensity (see Fig. 3b), whereas DNA-T would greatly enhance the fluorescence intensity (see Fig. 3c). This is because in the presence of DNA-R, probe 1 and probe 2 exist mainly in the dissociated form. However, when adding the target DNA-T, one side of probe 1 and probe 2 would hybridize with it. Simultaneously, both of the other side of probe 1 and probe 2 would be fixed in DNA-T and would be easier to form G-quadruplex. The G-quadruplex–berberine complex was formed and generated a strong fluorescence signal. Based on these observations, it suggests that the probes are able to detect DNA-T effectively and sensitively according to the fluorescence signal changes.

### 3.4. Calibration curve for DNA-T

Under the optimized conditions, a linear relationship was obtained between $\triangle F/F_R$ and the concentration of DNA-T in the range of $5.0\times10^{-9}$–$9.0\times10^{-7}$ mol/L (see Fig. 4). A linear regression equation of the calibration graph was set up:

$$F = 384.9 + 46.944\ C_{\text{DNA-}T} (R = 0.9978) \quad (1)$$

where $F$ is the fluorescence intensity at 527 nm, $C_{\text{DNA-T}}$ represents the concentration of DNA-T, and $R$ is the regression coefficient. As the concentration of DNA-T increases from $5.0\times10^{-9}$ mol/L to $9.0\times10^{-7}$ mol/L, the fluorescence intensity increase becomes

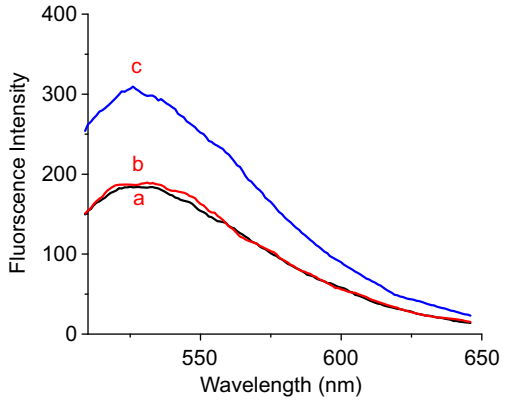


**Fig. 3.** Identification of DNA-T 0.05 mol/L Tris–HCl (pH=7.5) containing 0.1 mol/L KCl with berberine ($1\times10^{-5}$ mol/L) and (a) probe-1 and probe-2 ($5\times10^{-8}$ mol/L). (b) probe-1, probe-2 and DNA-R ($5\times10^{-8}$ mol/L). (c) probe-1, probe-2 and DNA-T ($5\times10^{-8}$ mol/L).

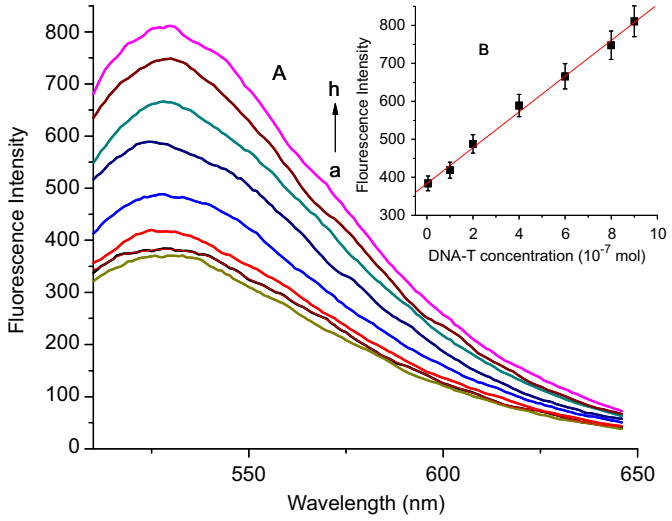


**Fig. 4.** Calibration curve for DNA-T 0.05 mol/L Tris–HCl (pH=7.5) containing 0.1 mol/L KCl with probes ($5\times10^{-7}$ mol/L) and berberine ($1.0\times10^{-5}$ mol/L). A: fluorescence curve with various concentration (mol/L): (a) 0.0 (b) $5.0\times10^{-9}$ (c) $1.0\times10^{-7}$ (d) $2.0\times10^{-7}$ (e) $4.0\times10^{-7}$ (f) $6.0\times10^{-7}$ (g) $8.0\times10^{-7}$ (h) $9.0\times10^{-7}$ B: linear relationship between DNA-T concentration and fluorescence intensity.

obvious. It is because in the presence of DNA-T, the probes boost up G-quadruplex formation and the fluorescence intensity is then enhanced remarkably The detection limit is estimated to be $2.0\times10^{-9}$ mol/L at a signal-to-noise of 3. It is calculated according to the following equation:

$$\mathrm{LOD}=3s/\sigma \quad (2)$$

where $s$ is the standard deviation which calculated from ten times for the blank determination (without DNA-T); $\sigma$ is the slope of the calibration curve.

## 4. Conclusion

In conclusion, we have developed a new and simple sensor using binary probes for the detection of GMO with good selectivity and sensitivity. We also demonstrated that in the presence of DNA-T, the sensor is able to fold into a G-quadruplex, which bind with berberine to generate a fluorescence signal. Based on the changes of fluorescence intensity, the DNA-T is able to be quantified in the range of $5.0\times10^{-9}$–$9.0\times10^{-7}$ mol/L with a low detection limit of $2.0\times10^{-9}$ mol/L.

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