An *E. coli* SOS-EGFP biosensor for fast and sensitive detection of DNA damaging agents

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Abstract

An *E. coli* SOS-EGFP biosensor which expresses enhanced green fluorescent protein as a reporter protein under the control of *recA* gene promoter in SOS response was constructed for detection of DNA damage and evaluation of DNA damaging chemicals. The chemicals that may cause substantial DNA damage will trigger SOS response in the constructed bacterial biosensor, and then the reporter *egfp* gene under the control of *recA* promoter is stimulated to express as a fluorescent protein, allowing fast and sensitive fluorescence detection. Interestingly, this biosensor can be simultaneously applied for evaluation of genotoxicity and cytotoxicity. The SOS-EGFP bacterial biosensor provides a sensitive, specific and simple method for detecting known and potential DNA damaging chemicals.

Key words: bacterial biosensor; detection; DNA damage; genotoxins; SOS response

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Introduction

DNA damage may occur at nucleotide level, strand level, chromosome level and genome level, and can be present as unusual modifications of nucleotides (alkylation, oxidation, adducts formation and abnormal methylation), strand break (single strand break and double strand break), crosslinking (inner-strand and intra-strand with DNA), micronucleus, abnormality of chromosome number and so on (Friedberg et al., 2006). DNA damage will affect DNA replication, repair and gene expression, and may be mutagenetic to cells and further carcinogenesis in organisms.

Various chemicals, endogenous genomic defects and environmental factors can cause damage to genomic DNA (Arshad et al., 2010; Park et al., 2005; Risom et al., 2003). DNA damaging agents include the existed materials on the earth, for example, the unstable isotopes, and newly found or manmade chemicals, such as anticancer drugs.

With the large-scale production and use of chemicals, the toxicology of newly-produced chemicals and possible by-products should be provided, particularly for genotoxicity, mutagenicity and carcinogenicity (Ohe et al., 2004). In practical application, the concentrations of some DNA damaging agents, such as germicide (hydrogen peroxide with concentration of 1.0 mol/L, Doughty, 1990) and antibiotics (mitomycin C with concentration of 1.5 mmol/L, Hamza et al., 2005) are required to be high enough to kill their targets (microbial infectors and cancer cells

respectively). However, these agents may also be harmful to the disinfecting operators or the normal organs of treated patients especially for a long period of use. In most cases, the potential DNA damaging agents are exposed at low concentrations but with a long period of time in the environment. Therefore, the detection methods of high-sensitivity, high-throughput and low cost are of intensive interest. A number of methods have been developed for detection of DNA damage and DNA damaging agents. High sensitive separation methods combined with qualitative analytical technologies have been utilized to detect the DNA damage in terms of unique biomarkers such as oxidative DNA damage and stable DNA adducts. These analytical methods include HPLC-EC (Rodríguez-Ariza et al., 1999), LC-GC-MS (Cooke et al., 2006), LC-MS/MS (Feng et al., 2008) and UPLC-MS/MS (Feng et al., 2009), ultrasensitive CE-LIF immunoassay (Wang et al., 2009, 2010) and ^{32}P-post labeling test (Dunn et al., 1987; Funk et al., 2010). Although they can quantify or quantitate the known DNA damage and/or DNA damaging agents, it is challenging for these methods to be applied to detection and screening of unknown and potential DNA damaging agents, more challenging, for genotoxic chemical mixture.

To detect unknown DNA damaging agents and to evaluate the related DNA damage potency, a biological response SOS response which may occur in various bacteria is exploited to develop a novel type of biosensor for this purpose. In prokaryotes, SOS response will occur when their cells suffering from substantial DNA damage. During normal growth, cells undergo little DNA damage and SOS genes are negatively regulated by LexA repressor protein.

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The LexA protein binds to a consensus sequence (the SOS box) in the promoter region for those SOS genes. When DNA damage arises, the DNA replication will be blocked at DNA damage sites. Therefore, large mounts of single-strand DNA will appear which needs more RecA protein to bind to. The resulted RecA-ssDNA filaments provide the activated form RecA protein which interacts with the LexA repressor to facilitate the LexA repressor's self-cleavage from the SOS promoters (Nelson and Cox, 2005; Friedberg et al., 2006). At the early stage of SOS response, RecA protein can enhance its amount significantly for several times. Because the amount of RecA protein is closely related with the activity of *recA* promoter, the reporter EGFP protein under the control of *recA* promoter can manifest the expression of RecA protein. The fluorescence of EGFP protein can easily be tested by a fluorometer, so the fluorescent intensity can representative the activity of *recA* promoter, further displaying the level of SOS response of cells treated by chemicals.

The expressed EGFP protein from reporter gene displays 35-times enhanced fluorescence signal over the wild type green fluorescent protein (wtGFP) due to the double mutation of Phe64Leu and Ser65Thr (Cormack et al., 1996). And the EGFP protein gets increased fluorescence intensity and photostability, enhanced 37°C folding efficiency and the same excitation and emission peaks with FITC, which makes more general researcher for practical use of EGFP protein (Heim et al., 1995; Cormack et al., 1996). In addition, the EGFP protein needs only oxygen to emit fluorescence without exogenous substrates or co-factors while enzymatic (such as beta-galactosidase) and *lux* reporters need reaction with other substrates to produce detectable signal with increasing cost, especially at large scale detection of chemicals (Miller, 1972; Vollmer et al., 1997).

In the present study, we constructed a bacterial biosensor in an *E. coli* strain with a transformed *egfp* gene as a reporter gene under the control of the promoter of *recA* gene, and developed an SOS-EGFP test. By this test, the biosensor cells treated by chemicals can produce brighter fluorescence than the untreated control if the chemicals can induce substantial DNA damage. We discard the complicated procedure of sample preparation and separation, and provide an easy, speedy and reproducible assay to screen the DNA damaging agents.

1 Materials and methods

1.1 Bacterial strains and culture conditions

All *E. coli* bacterial strains grew in the LB medium (pH 7.4). The LB medium was prepared with 10 g bacterial peptone (BD Biosciences, USA), 5 g yeast extract (BD Biosciences, USA), 5 g NaCl, 15–20 g agar if used for solid medium, and 1000 mL H_2O (containing 60 μg/mL ampicillin or 40 μg/mL kanamycin if necessary). For log phase bacteria culture, the bacterial mono-clone grown on the LB agar plate was inoculated into proper volume of LB liquid medium and was incubated at 37°C overnight.

After dilution (1:50, V:V) in fresh LB medium, the culture was incubated at 37°C until the optical density at 600 nm (OD_{600}) reached 0.2 to 0.4. This procedure may take about 2–3 hr at 130 r/min at 37°C. The bacterial strains and plasmids used in this study are listed in Table S1.

1.2 Molecular cloning and construction of BPE strain

E. coli BL21(DE3)/pETEGFP (BPE strain) was obtained by genetic engineering. The *egfp* gene was obtained from pEGFP-C1 plasmid by polymerase chain reaction (PCR) amplification. PCR amplification were performed by using a *Nde*I containing forward primer (5′-GGAATTCcatatgATGGTGAGCAAGGGCGAGGA-3′) and a *BamH*I containing reverse primer (5′-AACGggatccGAGCTCGAGATCTGAGTCC-3′). The amplification procedure was as follows: predenatured at 95°C for 4 min, 30 cycles with denaturation at 95°C for 45 sec, annealing at 55°C for 45 sec, extension at 74°C for 50 sec, with a final extension at 74°C for 5 min.

The pET-16b vector contained a T7 promoter, a multiple cloning site for expression of the cloned protein gene with a His-tag, and an ampicillin resistant gene for selection and amplification in *E. coli* cells. The pET-16b vector and a fragment of amplified *egfp* gene were double-enzymatic digested with *Nde*I and *BamH*I, and then the two fragments were ligated. The resulted pETEGEP vector was utilized for verification of EGFP protein expression in BL21 (DE3) cells. The positive transformants were screened by ampicillin resistant and confirmed by colonial PCR. Colonial PCR amplification was performed by using a forward primer (5′-TAATACGACTCACTATAGGGG-3′) and a reverse primer (5′-GCTAGTTATTG CTCAGCG-3′).

1.3 Molecular cloning and construction of B5 strain

E. coli BL21(DE3)/pETPrecAegfp5 (B5 strain) was obtained also by genetic engineering. The *E. coli* B5 strain expresses EGFP protein under the control of *recA* promoter. The fragment of *recA* promoter was amplified by PCR using *E. coli* K12 ER2925 genomic DNA as a template. The *recA* promoter is a DNA fragment of 250 bp long upstream of the *recA* gene open reading frame, including the ribosomal binding site and TATA box. PCR amplification was performed by using a *Bgl*II containing forward primer (5′-AGGAagatctGGCAGTGAAGAGAAGCCTGT-3′) and an *Nco*I containing reverse primer (5′-CATGccatggTTACTCCTGTCATGCCGGGT-3′). PCR was performed according to the following program: predenatured at 94°C for 3 min, then 30 cycles of 94°C for 45 sec, 55°C for 45 sec, 72°C for 30 sec, with a final 5 min elongation step at 72°C.

The pET-16b vector was digested with double-enzyme of *Bgl*II/*Nco*I to remove the T7 promoter and named pET-16b::P_{T7-} vector. Then the double-enzyme digested *recA* promoter was ligated into the pET-16b::P_{T7-} vector and the recombinant is named as pET-16b::P_{T7-}::P*recA* vector. The *egfp* gene from pEGFP-C1 plasmid was ligated into the *Nde*I/*BamH*I site of pET-16b::P_{T7-}::P*recA* vector. The ligated product of *egfp* gene and pET-16b::P_{T7-}::P*recA*

vector was transformed into the subcloning strain *E. coli* DH5a. The positive transformants were screened by ampicillin resistant and confirmed by colonial PCR. Colonial PCR amplification was performed by using a forward primer (5′-GGCAGTGAAGAGAAGCCTGT-3′) and a reverse primer (5′-GCTAGTTATTGCTCAGCG-3′). The PCR was performed as the following conditions: pre-denatured at 95°C for 4 min, 30 cycles with denaturation at 95°C for 45 sec, annealing at 52°C for 45 sec, extension at 72°C for 1 min, with a final extension at 72°C for 5 min. The recombined vector was extracted from *E. coli* DH5a and transformed into the protein-expressing strain *E. coli* BL21 (DE3).

PCR was performed using a MyCycler Personal Thermal Cycler (BioRad, USA). The synthesis of primers and the sequencing of insertion DNA fragments were performed in Sangon, Shanghai, China. Restriction enzymes were purchased from New England, Biolabs, Co. And DNA ligation was operated using Rapid DNA Ligation Kit (Fermentas, CA). And DNA (and plasmids) extraction, purification, digestion, transformation, electrophoresis and buffer preparation were performed according to the Molecular Cloning handbook (Sambrook and Russell, 2001) or the instructions of commercial kits (Tiangen, Co. Beijing, China).

1.4 Bacterial induction

Aliquots of BPE cells (0.18 mL) of steady phase were induced by isopropyl β-D-1-thiogalactopyranoside (IPTG) with 0, 0.1 and 0.2 mmol/L for verification of the expression and function of EGFP protein in *E. coli* cells.

Aliquots of B5 cells of log phase were used for the SOS-EGFP test. Aliquots (0.18 mL) of the log phase culture cells were transferred into 0.6 mL tubes, and 20 μL of appropriate concentrations of the test compound in aqueous solution was added. The 20 μL of distilled water was added to the control tubes. The cells were incubated and induced at 37°C for 2 hr at 130 r/min. Experiments were performed three times, and the standard errors between the experiments were determined.

1.5 Fluorescent microscopy analysis

The fluorescence images of bacterial cells were obtained by confocal laser scanning microscope consisted of a Leica DMI 6000B inverted microscope (100× oil immersion objective, 1.40 numerical aperture NA) and a Leica TCS SP5 confocal scanning system. The fluorescence of *E. coli* cells were excited by 488 nm line of an argon ion laser (4.5 mW), and the emission was detected by a photomultiplier tube (PMT) at 500 to 560 nm. The PMT voltage was set at 890 V. The bright-field images were acquired at the same time with a PMT gain of 340 V. Each image was taken from a physical dimension of 155 μm×155 μm.

1.6 Fluorescence spectral scanning

The *E. coli* cells were excited at 480 nm and the fluorescence was scanned from 500 to 600 nm in 96-well plate (Corning, USA) with spectral scanning multimode reader (Varioskan®Flash, Thermo, USA) at room temperature.

The cell density (OD_{600}) was determined simultaneously using the same device.

1.7 Chemicals for SOS-EGFP test

Typical genotoxins including mitomycin C (MMC), N-methyl-N′-nitro-N-nitrosoguanidine (MNNG), nalidixic acid (NA), formaldehyde (CH_2O) and hydrogen peroxide (H_2O_2) were tested. The following reagents with varied concentrations were used in the assay: 1 to 500 nmol/L MMC (Inalco, USA), 0.1 to 10 μmol/L MNNG (Accu-Standard, USA), 0.1 to 8 μmol/L NA (Sigma, USA), 10 μmol/L to 10 mmol/L H_2O_2 (Beijing Chemical Works, China) and 10 μmol/L to 1 mmol/L CH_2O (Sigma, USA). Furthermore, 1 to 100 μmol/L kanamycin sulfate (KAN) (Amresco, USA), 1 to 100 mmol/L acetone (AC) (Beijing Chemical Works, China), 1 to 100 mmol/L *n*-butanol (BUT) (Beijing Chemical Works, China) and 0.1 to 10 mmol/L phenol (PHE) (Beijing Chemical Works, China) were used as negative controls.

1.8 Data analysis

The OD_x/OD_0 is defined as the ratio of optical density at 600 nm (OD_{600}) of treated sample and untreated control sample at the same induction time point. The specific fluorescent unit (SFU) is calculated as the tested fluorescence intensity divided by OD_{600} of the same sample. Induction factor (F_i) is the ratio of SFU value of treated sample and the SFU value of untreated sample (SFU_x/SFU_0) (Norman et al., 2005). Though various criteria have been used to assess the genotoxicity of chemicals (Quillardet and Hofnung, 1993), we utilized induction factor (F_i) to evaluate the genotoxicity of tested chemicals. If the value of F_i reaches 2 or more at any concentrations, it means the tested compounds were considered to be DNA damaging agents or genotoxic. The low limit of detection is defined as the dose at which the induction factor reaches the value as twice as that of the background (Mersch-Sundermann et al., 1994). Experiments were performed at least three times, and the standard deviations between the experiments were determined.

2 Results and discussion

2.1 Characterization and analysis of the cloned *egfp* gene

As *egfp* gene expressed in pET vectors (Novagen) has the possibility to form inclusion body in the protein expression strain (Verkhusha et al., 2001; Cabanne et al., 2005), it is necessary to investigate if eukaryotic *egfp* gene can be well expressed and has right function in prokaryotic *E. coli* cells and whether pET-16b vector can be used for its expression. *egfp* gene from plasmid pEGFP-C1 was cloned through PCR amplification and subcloned into pET-16b. The resulted pETEGFP plasmid was transformed into *E. coli* DH5a for positive recombinant selection. All the randomly screened *E. coli* monocolonies from the LB (ampicillin) agar plates were examined by colonial PCR for exponential amplification of the inserted *egfp* fragment. Agarose gel

electrophoresis analysis of the amplified DNA products (Fig. 1) showed that it indeed appeared and had a right length (952 bp) as same as that of template fragment (total length of primers, *egfp* gene and plasmid fragment) while no right amplified product was observed for the blank vector, confirming the insertion of *egfp* gene into the vector. The inserts were further characterized by sequencing both strands of the vectors in the regions between T7 promoter primer site and T7 terminator primer site. The sequencing results clearly showed that the inserted fragment showed 100% homologue sequence with the template (omitting the primer modifications containing the restriction sites) and the right direction (Fig. S1), confirming the correct insertion of the *egfp* gene in pET-16b vector.

The pETEGFP vector was transformed into a high expression strain *E. coli* BL21(DE3) and the resultant cells was named as BPE strain. The cells grew to steady phase and were induced by IPTG. Through fluorescent microscopic observation, it was found that strong fluorescence signal could be detected and imaged from the BPE strain in response to the IPTG induction (Fig. 2). It is evident that the fluorescence of BPE cells enhanced significantly upon the increase of induction concentration of IPTG (Fig. 2). These results suggest the feasibility to express the EGFP protein in *E. coli* BL21(DE3). The observed fluorescence signal also reflects the fact that the expressed EGFP protein can be correctly folded in its native conformation. In the pET-16b vector a His-tag is connected with the N-terminal of recombinant protein. The observed strong fluorescence signal of EGFP may also indicate that the His-tag does not affect the conformation and function of the fused EGFP protein in the SOS-EGFP host bacterial strain.

2.2 Expression and function of the fused *recA* promoter-*egfp* gene

With the accomplishment of the correct expression and function of recombinant *egfp* gene in *E. coli* BL21(DE3) cells, we further investigate the construction of fused *recA* promoter-*egfp* gene in plasmid pET-16b and its expression and function in *E. coli* BL21(DE3) cells. Agarose gel electrophoresis analysis showed that the amplified *recA* promoter fragment can be detected in pET-16b::P$_{T7^-}$::P*recA* plasmid and has a correct length of 397 bp (total length of primers, *recA* promoter and plasmid fragments) through colonial PCR amplification (Fig. 3a). Note that in colonial PCR amplification, upstream primer of *recA* promoter of inserted fragment and T7 terminator primer on pET-16b plasmid were used for enhancing the specific amplification of inserted fragments in recombinant plasmids. The DNA fragment of fused *recA* promoter-*egfp* gene could be observed in pET-16b::P$_{T7^-}$::P*recA* vector (Fig. 3a) and also have a correct length of 1100 bp,
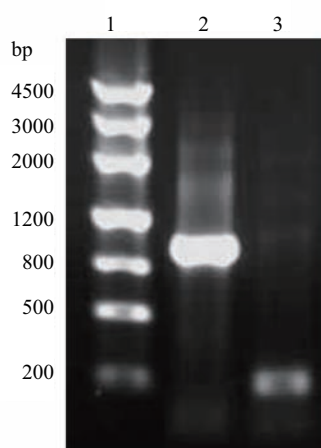

Fig. 1 Identification of inserted *egfp* gene in recombinant pETEGFP plasmid by agarose gel electrophoresis. Lanes 1 is DNA marker, and lanes 2 and 3 are amplified products by colonial PCR from positive recombinant pETEGFP vector and blank vector, respectively. Electrophoresis conditions: 0.8% agarose gel, 1× TBE buffer, 4 V/cm, 40 min.
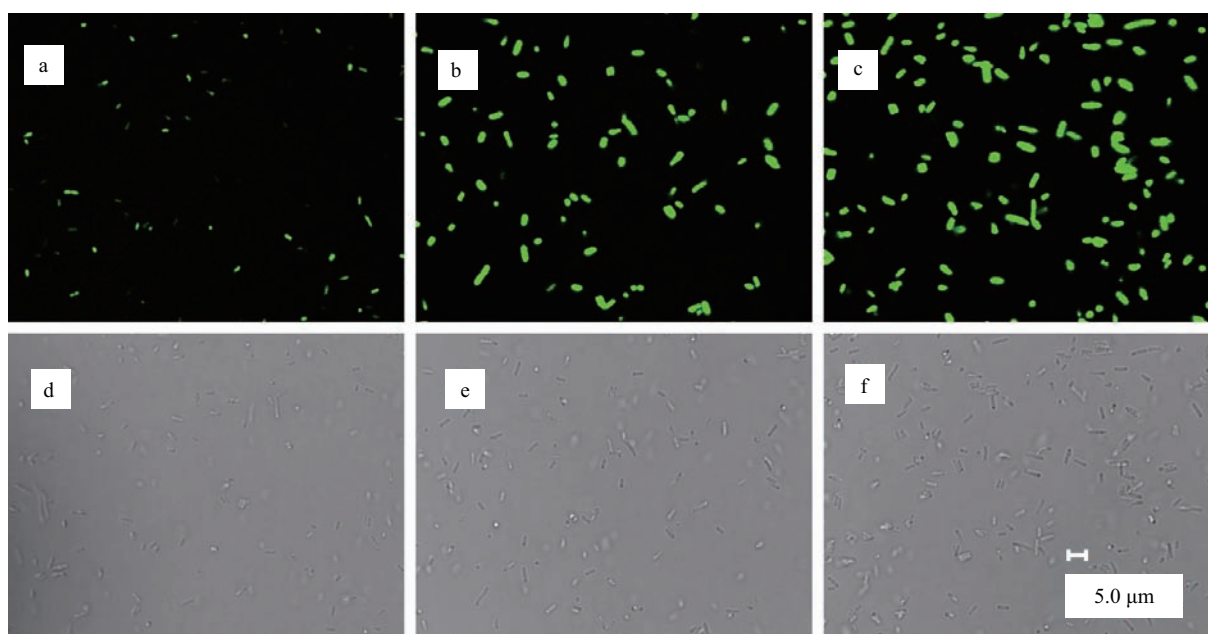

Fig. 2 Expression of *egfp* gene from pETEGFP vector in BPE cells upon IPTG induction. The BPE cells expressing EGFP protein were fluorescence imaged by confocal laser-scanning microscopy. The cells were not induced (a), or induced by 0.1 mmol/L IPTG (b) or 0.2 mmol/L IPTG (c). The corresponding images at bright field are shown in (d), (e) and (f), respectively.
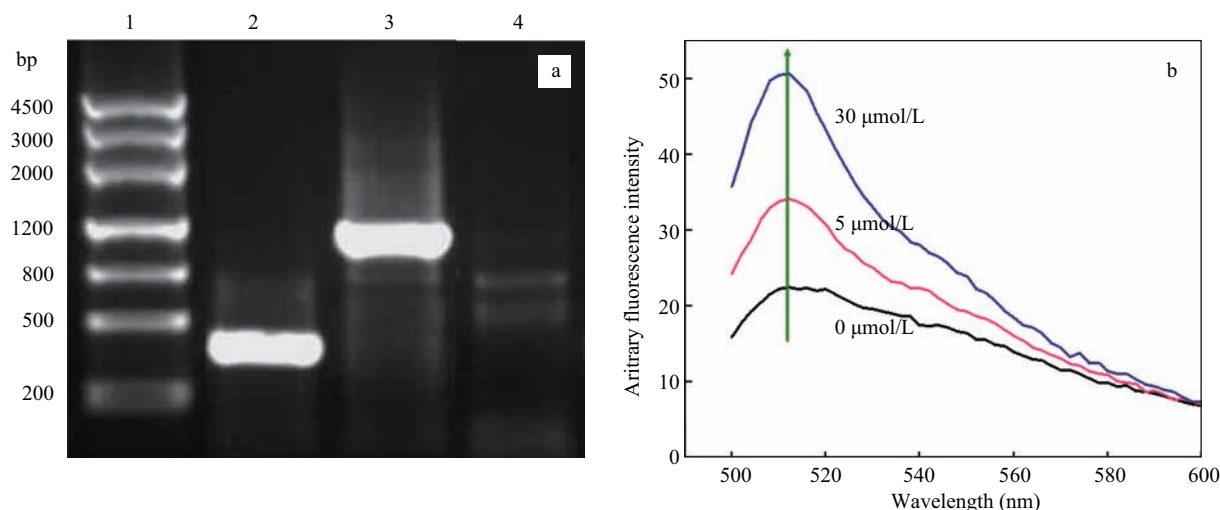
Fig. 3 Identification of function of the fused *recA* promoter-*egfp* gene in recombinant vector by agarose gel electrophoresis and fluorescence spectra scanning. (a) DNA marker/bp (lane 1), *recA* promoter in pET-16b::P_{T7-}::P*recA* vector (lane 2), and fused *recA* promoter-*egfp* in pET-16b::P_{T7-}::P*recA*::*egfp* vector (lane 3), blank vector (lane 4). Electrophoresis conditions: 0.8% agarose gel, 1× TBE buffer, 4 V/cm, 40 min. (b) B5 strain cells grew to log phase and were induced by 0, 5 μmol/L and 30 μmol/L NA at 37°C for 2 hr and the fluorescent spectra were scanned by excitation at 480 nm and emission at 500–600 nm.

confirming the insertion of fused *recA* promoter-*egfp* in the plasmid. The inserts were further characterized by sequencing both strands of the vectors in the regions between the upstream primer site of *recA* promoter and T7 terminator primer site. The sequence of *recA* promoter inserted into the pET-16b::P_{T7-}::P*recA* vector showed 99.20% similarity to the *recA* promoter from the *E. coli* ER2925 genome DNA sequence and the right direction (Fig. S2). A fragment in the sequence of *recA* promoter has been found previously to enhance protein translation in the *E. coli* cells (Thanaraj and Pandit, 1989). Moreover, the key fragment of cloned *recA* promoter (TATA box and RBS) is 100% homology with the *recA* promoter from the genome of *E. coli* ER2925 strain. The sequence of *egfp* gene inserted into the pET-16b::P_{T7-}::P*recA* vector showed 99.88% homology to the *egfp* gene from the pEGFP-C1 plasmid (omitting the primer modifications containing the restriction sites) and the right direction (Fig. S3).

In order to confirm whether the fused *recA* promoter-*egfp* gene can be correctly expressed and functioned, the *E. coli* BL21(DE3)/pETPrecAegfp5 (B5 strain) was first induced by a well-known DNA damaging agent nalidixic acid (NA), which can cause DNA strand break by inhibition of bacterial DNA topoisomerase II and prevention of DNA replication (Newmark et al., 2005). Then the fluorescence spectra of untreated and treated B5 cells were scanned. In contrast with untreated B5 cells, the fluorescence intensity of B5 strain cells treated by NA increased sharply with the increase of NA concentrations (Fig. 3b). All these results indicate that *recA* promoter inserted into the plasmid can respond to substantial DNA damage and the fused *egfp* gene is under the control of *recA* promoter, leading to highly elevated expression of EGFP protein in SOS response.

2.3 Detection of toxicity of chemicals by B5 strain cells

Figure 4 confirms that B5 cells demonstrated elongation morphology contrast with untreated B5 cells through 2

μmol/L NA treatment at 37°C and 2 hr at 130 r/min. The *E. coli* cells stopped cellular septation and formed filament as a consequence of the SOS response to DNA damage (Huisman et al., 1984). Therefore, we can conclude that B5 cells triggered SOS response to NA treatment which caused substantial DNA damage to B5 cells. At the same time, B5 cells which were treated by 10 mmol/L phenol (a negative control), did not show significant cellular filament (data not shown).

For toxicity detection, the treated cells were scanned immediately after induction for recording the fluorescence spectra and optical density in 96-well plate in a microplate reader at room temperature. When B5 cells were treated by various concentrations of nalidixic acid and phenol, the induction factor and optical density ratio (OD_x/OD_0) curves were surveyed to identify the toxicity of the two chemicals to our constructed biosensor. As it was identified by Quillardet and Hofnung (1993), if the value of F_i reaches 2 or more at any concentrations, it means the tested compounds were considered to be DNA damaging agents or genotoxic. And it was shown in Fig. 5 that the values of F_i of nalidixic acid which can cause DNA strand break by inhibition of DNA replication were over 2 since the induction concentration of 1.83 μmol/L, at which the fluorescence intensity doubled contrast with the background fluorescence intensity. The low limit of detection (1.83 μmol/L) of B5 strain to nalidixic acid was lower than that of reported biosensors (3.57 μmol/L in Kostrzynska et al., 2002 and 4.6 μmol/L in Ptitsyn et al., 1997). During the tested concentration from 0.1 to 8.0 μmol/L of nalidixic acid, the ratio of optical density of treated B5 cells declined with the increase of induction concentration, demonstrating a significant cytotoxicity to B5 cells. With 8 μmol/L nalidixic acid treatment, B5 cells had a cell viability of 59.4%. When B5 cells were treated by phenol, which was reported to target protein only, the induction factors never exceeded value of 2 even at 10 mmol/L of induction concentration, demonstrating the
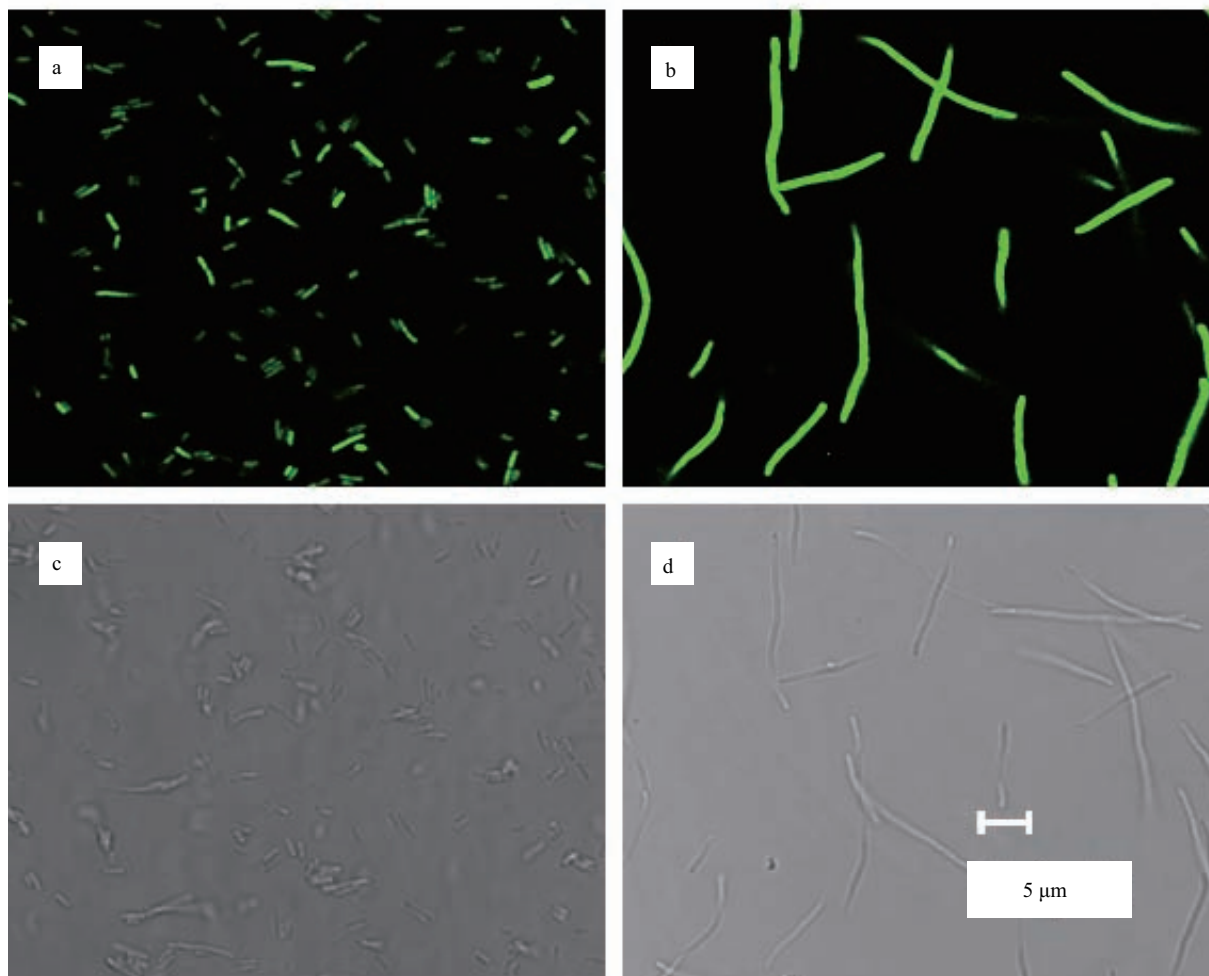
Fig. 4 Microscopic photos of B5 cells with nalidixic acid treatment. The B5 cells were imaged by confocal laser-scanning microscopy immediately after induction. The cells were not induced (a), or induced by 2 μmol/L NA (b) at 37°C for 2 hr at 130 r/min. The corresponding images at bright field are shown in (c) and (d), respectively.
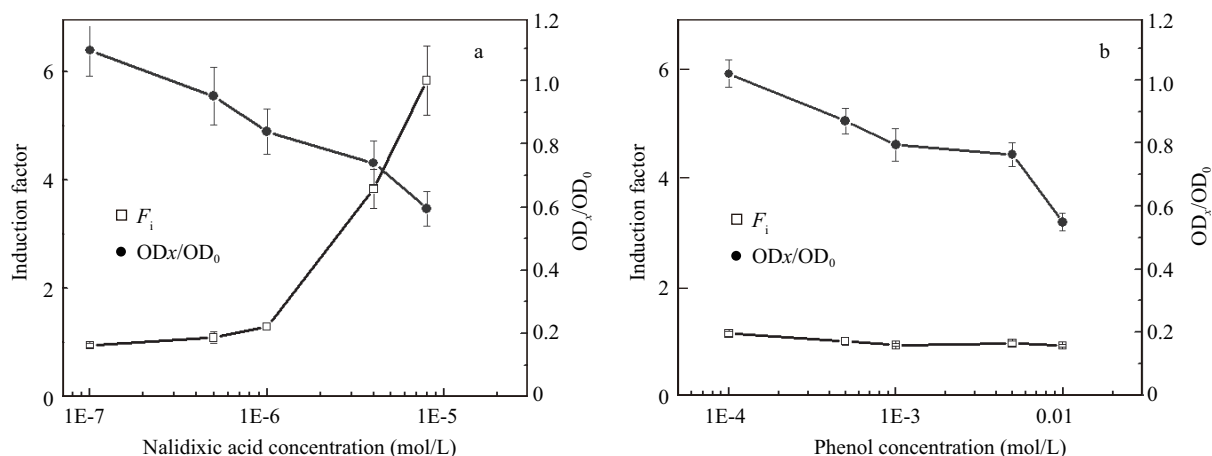
Fig. 5 Response of B5 strain to DNA damaging nalidixic acid and DNA damage-irrelevant chemical phenol. The induction factor (F_i) curves and the optical density ratio (OD_x/OD_0) curves of SOS-EGFP test using B5 cells to nalidixic acid (a) and phenol (b). Experiments were performed at least three times, and the standard errors between the experiments were determined.

irrelevant ability of DNA-damaging. At the same time, phenol had cytotoxicity to our biosensor during the tested concentration from 100 μmol/L to 10 mmol/L. In conclude, B5 strain biosensor could respond to genotoxin nalidixic acid for both genotoxicity and cytotoxicity, while showed only cytotoxicity to non-genotoxin phenol.

2.4 Applications of SOS-EGFP test

The constructed B5 strain which contained fused *recA* promoter-*egfp* gene was further applied to evaluation and screening of DNA damaging agents. The B5 cells were incubated to log phase and aliquots of culture were suffered

from chemical treatment for 2 hr at 130 r/min at 37°C.

Other genotoxins, including MMC, MNNG, CH$_2$O and H$_2$O$_2$ which caused DNA damage by different mechanism (Table S2), were tested using our B5 biosensor cells. The induction factors of B5 cells displayed dose-dependent response to these DNA damaging agents (Fig. 6a), manifesting its potential for detecting a large mount of chemicals with different DNA-damaging mechanism. This is consistent with the previous bacterial biosensors (Ptitsyn et al., 1997; Kostrzynska et al., 2002), further demonstrating the reliability of our constructed biosensor. These genotoxins showed a dose-response relationship with the increase of induction concentrations. In the tested concentrations of the genotoxins, H$_2$O$_2$ showed a induction factor decrease at higher concentration, indicating at which the cytotoxicity effect exceeded the genotoxicity effect to the B5 cells.

The concentrations of low limit of detection were varied from nanomolar (MMC) to micromolar (MNNG and NA) or millimolar (CH$_2$O and H$_2$O$_2$) concentrations, which were several orders of magnitude lower than that of practical dosages (Table S2). These results indicated that the constructed B5 strain could sensitively respond to the DNA damaging agents at low dose. In contrast, the typical non-genotoxic chemicals without DNA damaging potency (Table S3), including acetone, *n*-butanol, phenol and kanamycin, got the F_i values of less than 2 even at high concentrations. These non-genotoxic chemicals failed to induce SOS response in B5 cells (Fig. 6a), confirming the specificity of the B5 test against DNA damaging agents. Note the response of B5 cells to kanamycin. Although as a aminoglycoside antibiotic kanamycin can not damage DNA structure, kanamycin inhibits the expression of proteins in bacteria. Kanamycin could trigger the induction process to a value of near 2 at induction concentration of 50 μmol/L. That is because kanamycin could induce the expression of RecA protein by the increase of "alarmone" synthesis which played the "trigger" role in derepression of SOS-operon (Kiselev, 1987).

The OD$_x$/OD$_0$ value represents the percentage of survived cells (cell viability) after chemical treatment.

Though dead or non-dividing cells also contribute to optical density, the value of OD$_x$/OD$_0$ can display the cytotoxic potential of tested chemicals. Whatever to the known genotoxins or to the negative chemicals, all the tested chemicals in the tested concentration with an exception of acetone could cause a sharply decline in the value of OD$_x$/OD$_0$ with the increasing concentration (Fig. 6b), indicating most of tested chemicals had cytotoxic effect to B5 strain cells. The values of OD$_x$/OD$_0$ of our B5 cells, which were 0.51 (to 0.5 μmol/L MMC), 0.31 (to 10 μmol/L MNNG) and 0.63 (to 1 mmol/L CH$_2$O), demonstrated a much lower value than that of the *E. coli* C600 (pPLS-1) cells, a bacterial biosensor constructed in SOS *lux* test (Ptitsyn et al., 1997), which were 0.88 (to 1 μmol/L MMC), 0.94 (to 10 μmol/L MNNG) and 0.80 (to 1 mmol/L CH$_2$O). Our SOS-EGFP bacterial biosensor was more sensitive than the SOS *lux* bacterial biosensor against the relative optical density (OD$_x$/OD$_0$).

Despite of no observation of DNA damage caused by them, it is interesting that these non-genotoxic chemicals display detectable cytotoxicity, consistent with their target at proteins in organisms (Fig. 6b). These chemicals cannot react with DNA, and therefore cause no DNA damage in the SOS-EGFP test strain. Therefore, these chemicals will only display cytotoxicity in the B5 strain but no genotoxicity. These results further prove that the chemical caused cytotoxicity of B5 strain cannot interfere with the measurement of the DNA damage effect, e.g., especially for SOS response to DNA damage.

This observation indicates the constructed B5 strain can be used to test cytotoxicity of the chemicals while measuring the DNA damage potency. Due to simultaneous test on DNA damage related genotoxicity and DNA damage-independent cytotoxicity, B5 strains may be potentially useful to evaluate the relative potency of chemicals in genotoxicity and cytotoxicity.

The SOS-EGFP test using our B5 biosensor, had the advantages of SOS-reporter genotoxicity/mutagenesis test. For example, it provided a detection method *in vivo* not disturbing the living cells and in a short time (2 hr); large scale of chemicals with different mechanisms to damage
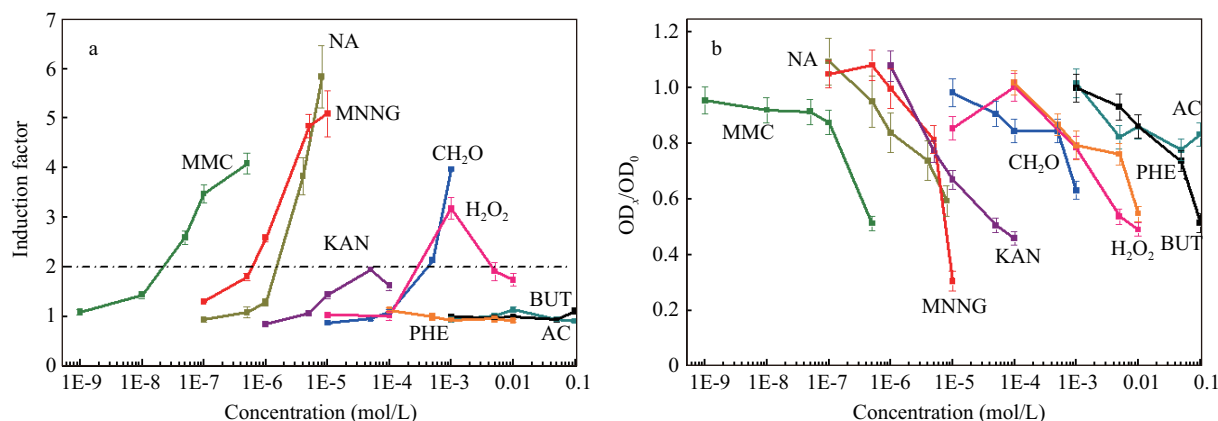
Fig. 6 Response of B5 strain to DNA damaging genotoxins and DNA damage-irrelevant chemicals. (a) the induction factor (F_i) curves of SOS-EGFP (B5 cells) test; (b) the optical density ratio OD$_x$/OD$_0$ of B5 cells. Experiments were performed at least three times, and the standard errors between the experiments were determined. DNA damaging genotoxins: MMC: mytomycin C, MNNG: N-methyl-N'-nitro-N-nitrosoguanidine, NA: nalidixic acid, H$_2$O$_2$: hydrogen peroxide, CH$_2$O: formaldehyde. DNA damage-irrelevant chemicals (negative controls): AC: acetone, BUT: *n*-butanol, PHE: phenol, KAN: kanamycin.

DNA can be detected by the same strain comparing with Ames test which need lots of strains to detect specific kind of chemicals (Ames, 1979). In SOS-reporter systems, SOS *lux* test needs substrate addition for the chemical luminescence detection. And when a wide range of chemicals needed detection, the cost of the SOS *lux* test will enhance significantly; and this will restrict the practical application of SOS *lux* test (Ptitsyn et al., 1997). SOS-GFPmut3 test (Kostrzynska et al., 2002) used the mutant of wild type GFP, GFPmut3, as a reporter which could emit brighter fluorescence than the wild type GFP. However, sensitive detection of DNA-damaging agents was obtained needing another incubation at 4°C for 2 hr to get matured and fluorescent GFPmut3 after induction process of 90 min. Our biosensor using B5 strain needs no other substrate and more time to mature the fluorescent chromophore without loss of sensitivity and specificity.

3 Conclusions

Here we demonstrated the construction of a sensitive and specific bacterial biosensor (B5 strain) for detection of DNA damage and DNA damaging agents. It expects that the constructed biosensor can be potentially useful to detect and screen environmental carcinogen and anti-cancer drugs. Interestingly, the constructed biosensor is probably useful to simultaneously evaluate the genotoxicity and cytotoxicity.

Acknowledgments

Supplementary data

Supplementary data associated with this article can be found in the online version.

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Supplementary materials

Table S1 List of strains and plasmids used in this study

E. coli strains and plasmids	Relevant genotype and characteristicsa	Source
E. coli strains		
DH5a	F⁻ endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80dlacZΔM15 Δ(lacZYA-argF)U169, hsdR17(r_{K-} m_{K+}), λ⁻; host strain modified for optimal plasmid yield.	Tiangen, Co., Beijing
K12 ER2925	ara-14 leuB6 fhuA31 lacY1 tsx78 glnV44 galK2 galT22 mcrA dcm-6 hisG4 rfbD1 R(zgb210::Tn10)TetS endA1 rpsL136 dam13::Tn9 xylA-5 mtl-1 thi-1 mcrB1 hsdR2; strain for template of *recA* promoter.	New England Biolab, Co.
BL21(DE3)	F⁻ ompT gal dcm lon hsdSB(r_{B-} m_{B-}) λ (DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5]); host strain modified for optimal protein yield.	Tiangen, Co., Beijing
BPE	BL21(DE3) strain carrying pETEGFP plasmid; for verification of the EGFP protein expression in *E. coli* cells	This study
B5	BL21(DE3) strain carrying pETPrecAegfp5 plasmid; functional strain	This study
Plasmids		
pUC19	P_{lac} lacZ_a Ampʳ with a multiple cloning site	Tiangen, Co., Beijing
pEGFP-C1	pUC_{ori} P_{CMV} IE EGFP Kanʳ; template of *egfp* gene	BD Biosciences Clontech
pET-16b	P_{T7} Ampʳ with a multiple cloning site, plasmid for protein expression	Novagen, Germany
pETEGFP	pET-16b plasmid containing *egfp* between *Nde*I and *BamH*I site	This study
pETPrecAegfp5	pET-16b/P_{T7-} encoding *egfp* gene under control of *recA* promoter	This study

Table S2 Part of toxicological information of DNA damaging chemicals tested in this study

Genotoxins	Characteristic	Mechanisms of action to biomacromolecules	Actual working concentration	LOD in this study
MMC	Anticancer drug	Inhibition of DNA depolymerization, induction of crosslinks during DNA replication, cessation of DNA replication (Sjöbloma et al., 1995)	1.5 mmol/L (Hamza, 2005)	50 nmol/L
MNNG	Alkylated agent	Formation of O^6-methylguanine O^6-meG (Roos et al., 2004), which can pair with cytosine (C) or thymidine (T) (Wang and Edelmann, 2006)	0.34–1.36 mmol/L (Arsha et al., 2010)	0.5 μmol/L
NA	Bacterial antibiotic	Inhibition of bacterial DNA topoisomerase II, prevention of DNA replication (Newmark et al., 2005)	215 μmol/L (Goss et al., 1965)	4 μmol/L
CH₂O	Aldehyde	DNA strand crosslink (Huang and Hopkins, 1993), single strand breaks (de Graaf et al., 2009)	13 mol/L (Power, 1995)	0.5 mmol/L
H₂O₂	Oxidizing agent	Oxidative DNA damage (Park et al., 2005)	1.0 mol/L (Doughty, 1990) 12 mol/L (Sulieman et al., 2004)	1 mmol/L

Low detection limit (LOD) is defined as the dose at which the induction factor reaches the value as twice as that of the background (Mersch-Sundermann et al., 1994).

Table S3 Part of toxicological information of non-genotoxins tested in this study

Chemicals	Characteristic	Mechanisms of action to biomacromolecules
Acetone	Organic solvent	Deposition of proteins (Simpson and Beynon, 2010)
n-Butanol	Organic solvent	Reaction with hydrophobic proteins (Cabello, 1980)
Phenol	Organic solvent	Change of secondary structure and deposition of proteins (Sambrook and Russell, 2001)
Kanamycin	Antibiotic	Inhibition of protein synthesis of bacteria and mycoplasma (Benveniste and Davies, 1973)

1	ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGAC
1	ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGAC

61	GGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTAC
61	GGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTAC

121	GGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACC
121	GGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACC

181	CTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAG
181	CTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAG

241	CAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTC
241	CAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTC

301	TTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTG
301	TTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTG

361	GTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCAC
361	GTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCAC

421	AAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAAC
421	AAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAAC

481	GGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCC
481	GGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCC

541	GACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCAC
541	GACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCAC

601	TACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTC
601	TACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTC

661	CTGCTGGAGTTCGTGACCGCCGCCGGGGATCACTCTCGGCATGGACGAGCTGTACAAGTCC
661	CTGCTGGAGTTCGTGACCGCCGCCGGGGATCACTCTCGGCATGGACGAGCTGTACAAGTCC

721	GGACTCAGATCTCGAGCTCAAGCTTCGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGA
721	GGACTCAGATCTCGAGCTCAAGCTTCGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGA

781	TCCACCGGATCTAGATAACTGATCA
781	TCCACCGGATCTAGATAACTGATCA

Fig. S1 Sequence of inserted *egfp* fragment in pETEGFP vector is homologue with the template of *egfp* sequence and has the same direction. Multiple alignment result was saved as two sequence alignment format. Alignment of *egfp_*sequence_insert (upper line) and *egfp_*sequence (lower line); identity = 100.00% (805/805); gap=0.00 (0/805).

```
  1    CAT TT TTACTCC TG TCATGCC GG GTAATAC CG GATAGTC AA TATGTTC TG TTGAAGC AA T
       | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
  1    CAT TT TTACTCC TG TCATGCC GG GTAATAC CG GATAGTC AA TATGTTC TG TTGAAGC AA T


 61    TAT AC TGTATGC TC ATACAGT AT CAAGTGT TT TGTAGAA AT TGTTGCC AC AAGGTCT GC A
       | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
 61    TAT AC TGTATGC TC ATACAGT AT CAAGTGT TT TGTAGAA AT TGTTGCC AC AAGGTCT GC A


121    ATG CA TACGCAG TA GCCTGAC GA CGCACCG CA TCACGGT CG CCGCTGA AG CATTCCC GCC
       |   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |   | | | | | |
121    A . GCA TACGCAG TA GCCTGAC GA CGCACCG CA TCACGGT CG CCGCTGA AG CAT . CCC GCC


181    GGG TA ATGCCTT CA CCGCGGG CA GTGGCAA AA GCAAACC AG ACGGTGC CG ACAGGCT TC T
       | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
179    GGG TA ATGCCTT CA CCGCGGG CA GTGGCAA AA GCAAACC AG ACGGTGC CG ACAGGCT TC T


241    CTT CACTGCC
       | | | | | | | | |
239    CTT CACTGCC
```

Fig. S2 Sequence of inserted *recA* promoter fragment in pET-16b::P$_{T7^-}$::P*recA* is homologue with the template of *recA* promoter sequence and has the same direction. Multiple alignment result was saved as two sequence alignment format. Alignment of RECA sequencing (upper line) and *recA* promoter template (lower line); identity = 100.00% (248/248); gap = 0.80% (2/250).

Fig. S3 Sequence of inserted *egfp* fragment in pETPrecAegfp5 vector is homologue with the template of *egfp* sequence and has the same direction. Multiple alignment result was saved as two sequence alignment format. Alignment of EGFP_Sequence_template (upper line) and EGFP sequencing (lower line); identity = 100.00% (805/805); gap = 0.12% (1/806).

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