Peer-reviewed version available at Sensors 2018, 18, 2823; doi:10.3390/s18092823

The Applications of Promoter-Gene Engineered Bio-Sensors

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Abstract:

Promoter is a small region of DNA sequence in response to various transcription factors, which initiates a particular gene expression. The promoter-engineered bio-sensor can activate or repress gene expression through transcription factor recognizing specific molecules, such as polyamine, sugars, lactams, amino acids, organic acids or redox molecule, however, the reported applications of promoter enhanced bio-sensor are not too much. This review paper highlights the strategies of construction of promoter-gene engineered bio-sensor with human and bacteria's genetic promoter array for high-throughput screening (HTS) molecular drugs, study of membrane protein's localization and nucleocytoplasmic shuttling mechanism of regulating factor, enzyme activity, detection of the toxicity of intermediate chemicals, and probing bacteria density to improve value-added product titer. These bio-sensors's sensitivity and specificity can be further improved by proposed approaches of Mn^{2+} and Mg^{2+} added random Error-prone PCR and site-directed mutagenesis which is applied for construction of bacteria's "mutant library". It is expected to establish flexible HTS platform (Bio-sensor array) to large-scale screen transcription factor-acting drugs, reducing the toxicity of intermediate compounds, and constructing gene dynamic regulatory system in "push and pull" mode to effectively regulate the valuable medicinal product production. This proposed novel promoter-engineered biosensors aided synthetic genetic circuit construction will maximize the efficiency of bio-synthesis of medicinal compound, which will greatly promote the development of microbial metabolic engineering and biomedical science.

Key words: bio-sensor; promoter; sensitivity; specificity; high-throughput screening (HTS); genetic promoter chip; "push and pull" mode; toxicity

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1. Introduction

With the development of life sciences and DNA molecular technology, we can discover the key gene, new enzyme or protein which controls the main signaling pathway for synthesis of desired product. Many genes or enzymes are directly regulated by transcription factor without effectors binding, however, some of genes not only need transcription factor, but also need compound effectively binding for dynamically controlling gene expression[1,2]. Access to large of quantities of these dynamic regulatory components is critical for the discovery of new bio-sensors for novel applications[3-5]. Therefore, promoters, transcription factors and molecular effectors collectively contribute to the discovery of bio-sensors and cause our much attention (**Table 1**).

Table 1. Promoters, transcription factor and molecular effectors are engineered for construction of bio-sensors

Engineered component	Approaches	Reference
Engineered promoter	An oleic acid biosensor replacing the native FadR-regulated *fadBA* promoter with a synthetic two copies of promoter into the strong phage *T7* pomoter.	[5][9] [10]
Engineered transcription factor	L-arabinose-responsive transcription factor engineered to specifically respond to the level of D-arabinose, acid lactone, and mevalonate.	[5]
Engineered molecular effectors	(1) Fluorescence of strain HF19 harboring P_{BAD}-*gfpuv* reporter plasmid (Ppcc442) and expressing AraC-mev (Ppcc423-mev), in the presence of the indicated concentration of small molecule inducers ("effectors"), such as mevalonate, succinic acid, L-arabinose, Triacetic acid lactone. (2) MphR inducers are macrolides, such as erythromycin, oleandomycin, nabomycin, pikromycin, methymycin, josamycin.	[4] [5]

1.1. Strategies for construction of promoter-enhanced bio-sensor

Because promoter is an important component for initiating gene expression, the strong promoters can be identified through comparative transcriptional analysis, which have high gene expression ratio. For the gene expression profile tell us that the up or down regulated genes which strong or weak promoters can be in responses to specific molecules, the strong promoter can be identified through microarray-based transcriptional analysis[6-8].

1.1.1. Synthesizing promoters for increasing their properties

Strong promoters can be selected according to comparative transcriptional analysis, and their strength can be characterized by fusing immunofluorescent protein, such as *mCherry* or *GFP* gene, for fluorescence testing, which enables us HTS screening transcription factor-acting molecular drugs. However, in most time, the activity of single promoter which is in responses to compound is weak and insufficient that cannot be effectively characterized, so the promoters to be synthesized to enhance their performance through certain DNA assemble technique, like DNA

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Brick method, which utilizes isocaudarner ligation for connecting of a series of promoters[9,10]. The approach of synthesizing promoters of *Corynebacterium glutamicum* from two directions are illustrated in **Figure 1**.

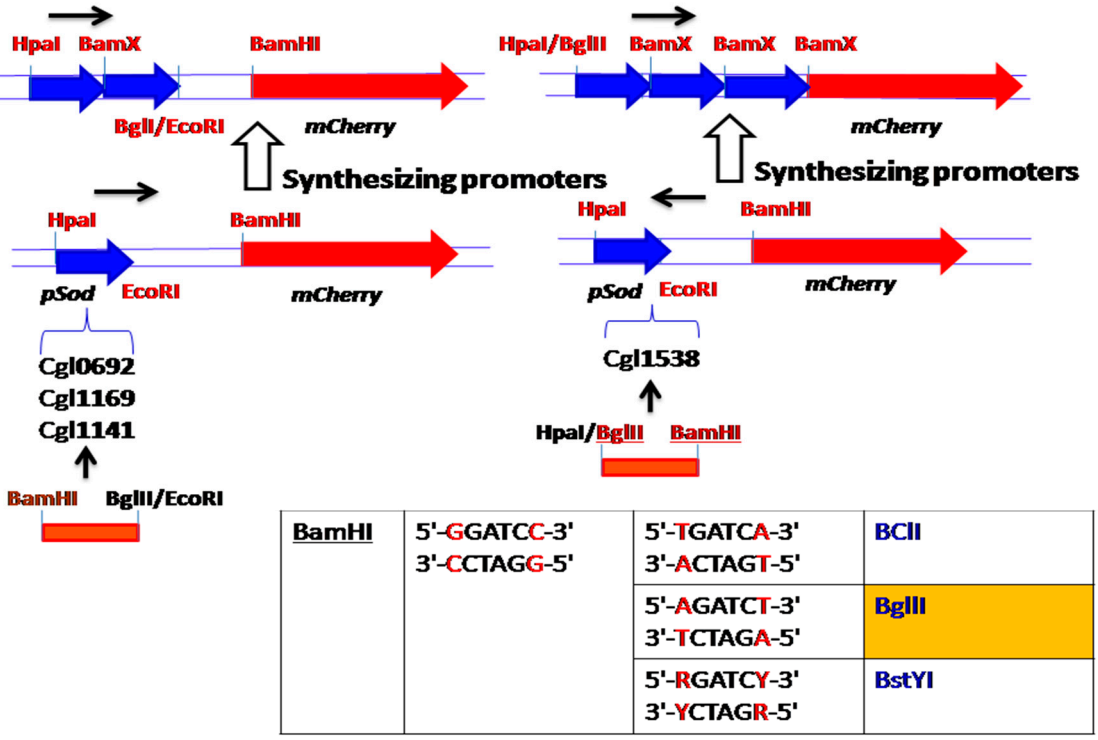

Figure 1. Synthesizing of promoters of *Corynebacterium glutamicum* by DNA Brick assemble technique with BglII/BamHI isocaudarner ligation from two directions.

1.1.2. Approaches of Mn^{2+} and Mg^{2+} added random Error-prone PCR and site-directed mutagenesis for further improving synthesized promoter's performance

The improvement of promoter's properties is beneficial for construction of sensitive bio-sensors. Although we can increase the strength of promoter by synthesizing them, how to further improve their sensitivity and specificity are needed our more consideration. Given that metal Mn^{2+} and Mg^{2+} added random Error-prone PCR random mutagenesis throughout the entire promoter's sequence can add random mutated sites into the promoter PCR fragments, which lead to change their performance, the mutants can be screened by Fluorescence Activated Cell Sorting analysis (FACS analysis) at various drug concentration[11], and the mutation sites can be identified by sequence analysis and can be validated by site-directed mutagenesis approach. Furthermore, site-directed mutagenesis approach could determine which mutation site contributed to the specificity of promoter. These newly introduced mutation sites are promoter's specific binding sites of transcription factor-molecular effector complex (**Figure 2A**) at the gene level. If the bacteria is infected by phage, the packaging phage can cause random mutations

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inserted into the TA sites of the bacteria gene's promoter[11,12] (**Figure 2B**).

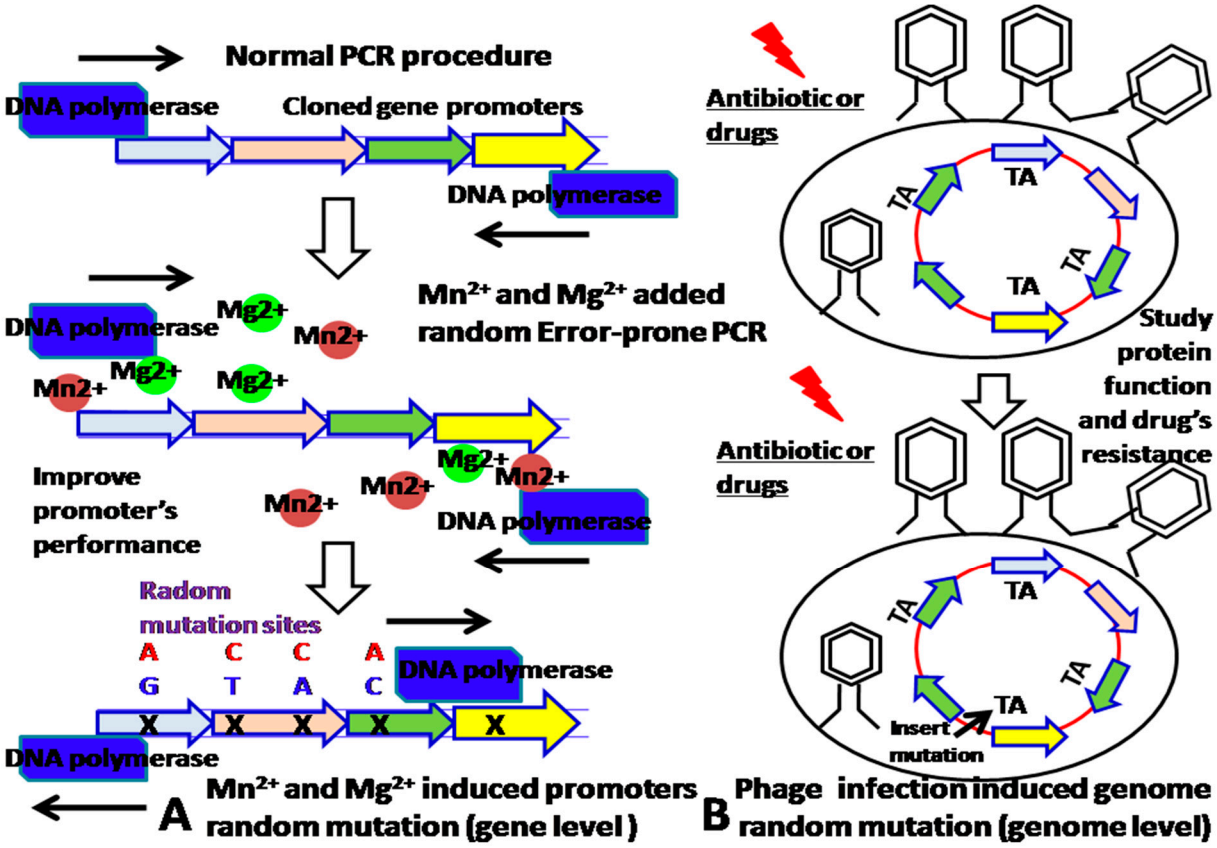

Figure 2. A. Mn^{2+} and Mg^{2+} added random Error-prone PCR approach introduces the gene mutation sites at the gene level. B. phage infection introduces genome random mutation sites.

2. Applications of Promoter-gene Engineered Bio-sensors

2.1. Construction of HTS platform for screening transcription factor (TF)-acting and anti-tumor drugs.

In many cases, bacteria triggers signaling cascades through transcription factor being in responses to specific compound for coping with environment. These transcription factors also play an important role in bacteria's physiological activities, such as regulation of salt and cell envelope stress that counteract extracytoplasmic stresses, NaCl and hormones balance, metal transporter protecting against oxidative stress, copper homeostasis, counteract hydrogen peroxide-mediated oxidative stress and gene expression inhibited by nitric oxide (NO)[13]. All these exhibited activities of bacteria are due to transcription factors are highly effective devices, which sensitively and specifically binding small compounds which trigger an allosteric responses to control the transcription of one or more genes. Because they sense various molecular effectors, transcription factors have been engineered to enable us to regulate valuable product production, including dicarboxylic acids, alcohols, phenylpropanoids, and lactone

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etc[14]. Nevertheless, the promoter still exhibits binding saturation effect and the gene expression is not very sufficient, which results in insufficiency of the drug screening. The strategy of synthesizing promoters, which will promote more TF-molecular effectors specifically binding gene promoter, will increase the DNA translation efficiency thus leading to the improvement of drug screening. So, promoter-enhanced bio-sensor and construction of genetic promoter chip of bacteria with synthesizing promoters enable us to construct HTS platform to screen transcription factor (TF)-acting compound, for example, we have screened up-regulated genes (Ratio≥6.0) of mutated *Corynebacterium glutamicum* (ER6937R42) that is correlated with highly producing L-Ornithine and up-regulated genes (Ratio≥7.0) of mutated *Corynebacterium glutamicum* (16-17-CPVF-ALE) that is correlated with highly producing putrescine[14,15]. The corresponding promoters can be found, assembled and fused with *mCherry or EGFP* gene for construction of HTS Platform for detecting drug L-Ornithine and putrescine [15](**Figure 3 and Figure 12**). We can further improve the sensitivity and specificity of these bio-sensors by site-directed mutagenesis approaches, to increase the sensitivity of detection of L-Ornithine and putrescine in bacteria. The method proposed can be extended to screening of other TF-acting molecular drugs or anti-tumor compound screening by cell array (**Figure 4**), especially those drugs associated with growth and energy metabolism of bacteria[16](**Figure 3**) or tumor growth and invasion[17,18] (**Figure 4**).

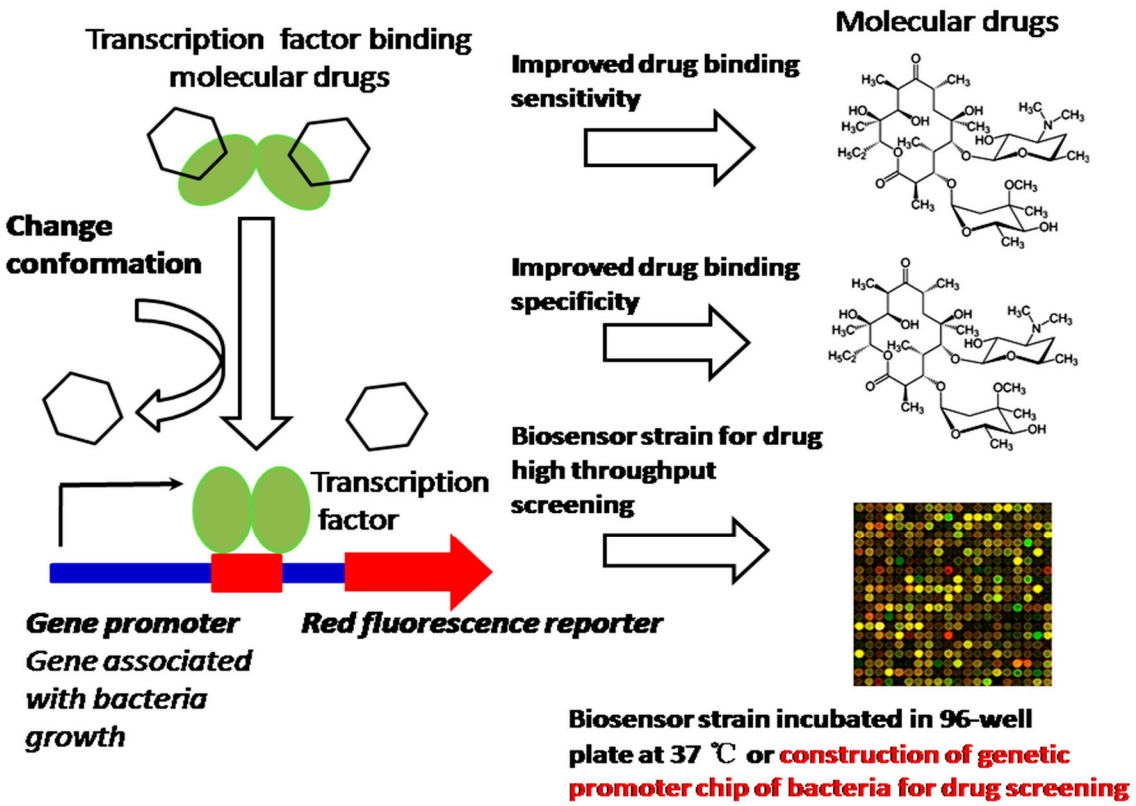

Figure 3. Construction of bio-sensor strain and genetic promoter chip of bacteria for HTS screening transcription factor (TF)-acting molecular drugs.

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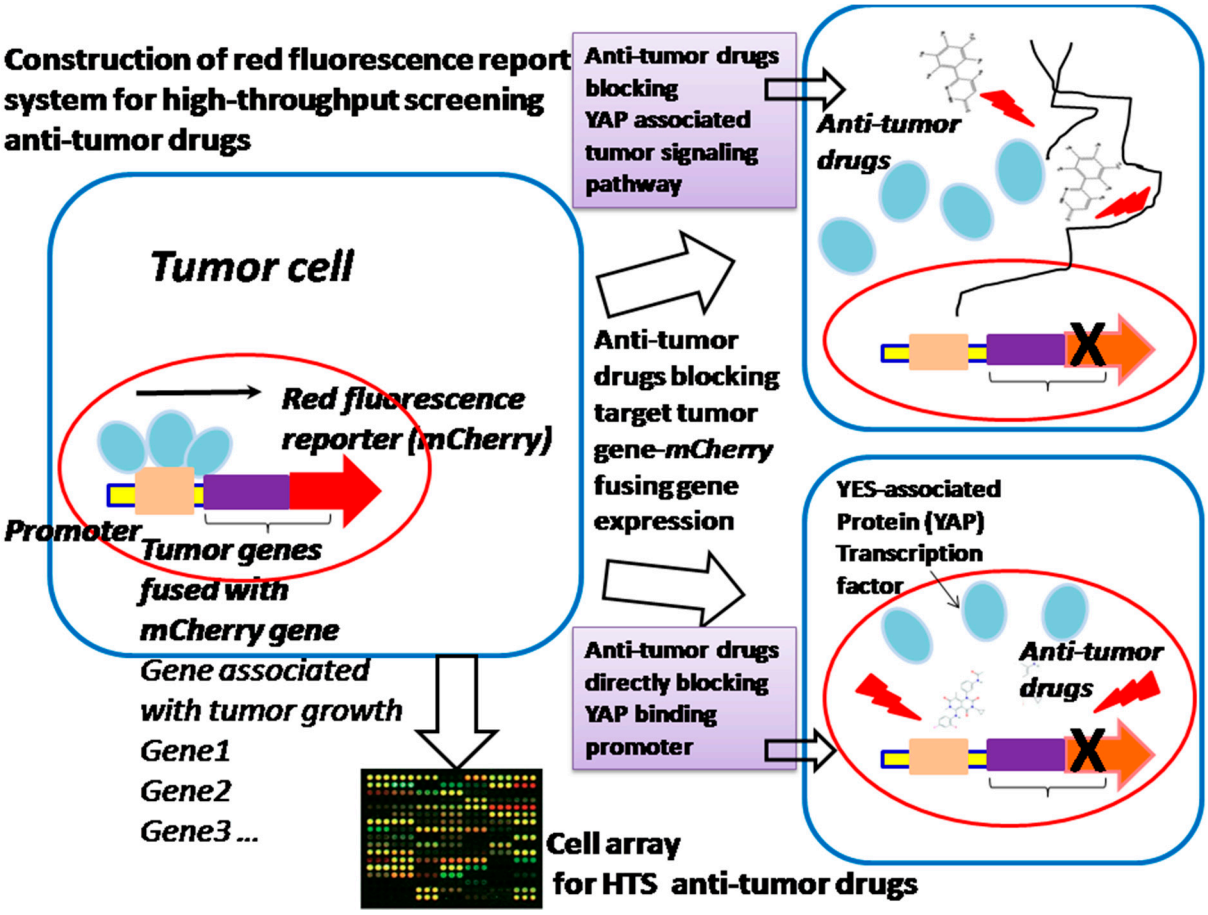

Figure 4. Construction of HTS platform (Cell array) for HTS anti-tumor drugs.

2.2. Performance enhanced promoter can be applied for studying the membrane protein's localization and enzyme activity

Due to the abundance of most membrane protein of bacteria on the cell surface is limited[19], the performance enhanced promoter can be cloned before *mCherry or EGFP* gene fused with membrane protein gene to increase the chimeric protein expression for studying membrane protein's localization and activity (**Figure 5**). Interestingly, if the *mCherry* gene and *EGFP* gene are fused with nucleocytoplasmic shuttling regulating factor YES-associated with transcription factor (YAP) which controls tumor growth and invasion, the YAP nucleocytoplasmic behavior of movement and localization can be observed and controlled[17,18]. So, the investigation of nucleocytoplasmic shuttling mechanism of YAP enable us to deep understand the tumorigenesis and oncology mechanism, and find more effective tumor therapy (**Figure 7**). Furthermore, if both *mCherry* and *EGFP* gene are fused with individual interactive proteins or enzymes, we can investigate the protein-protein interactions and bacteria-host cell interactions associated pathogenesis of infection diseases and mechanism of catalytical reaction of enzymes and

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their activity in cell array[20-23] (**Figure 5 and Figure 6**).

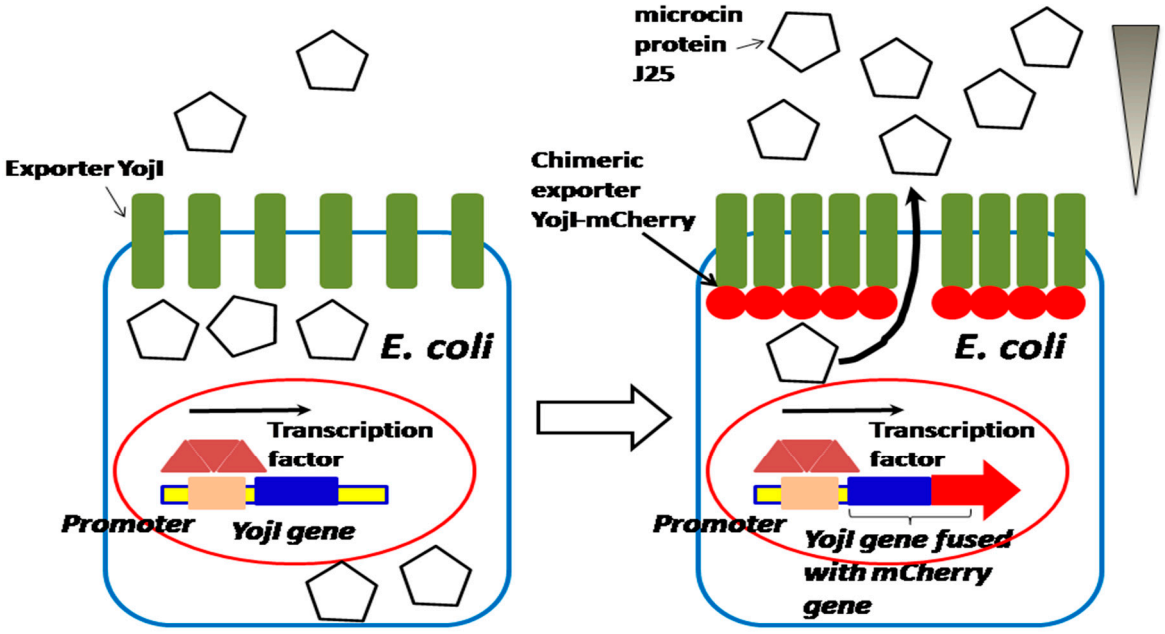

Figure 5. Performance enhanced promoter is applied for construction of chimeric exporter for studying the activity of YojI-mCherry of export of microcin protein J25.

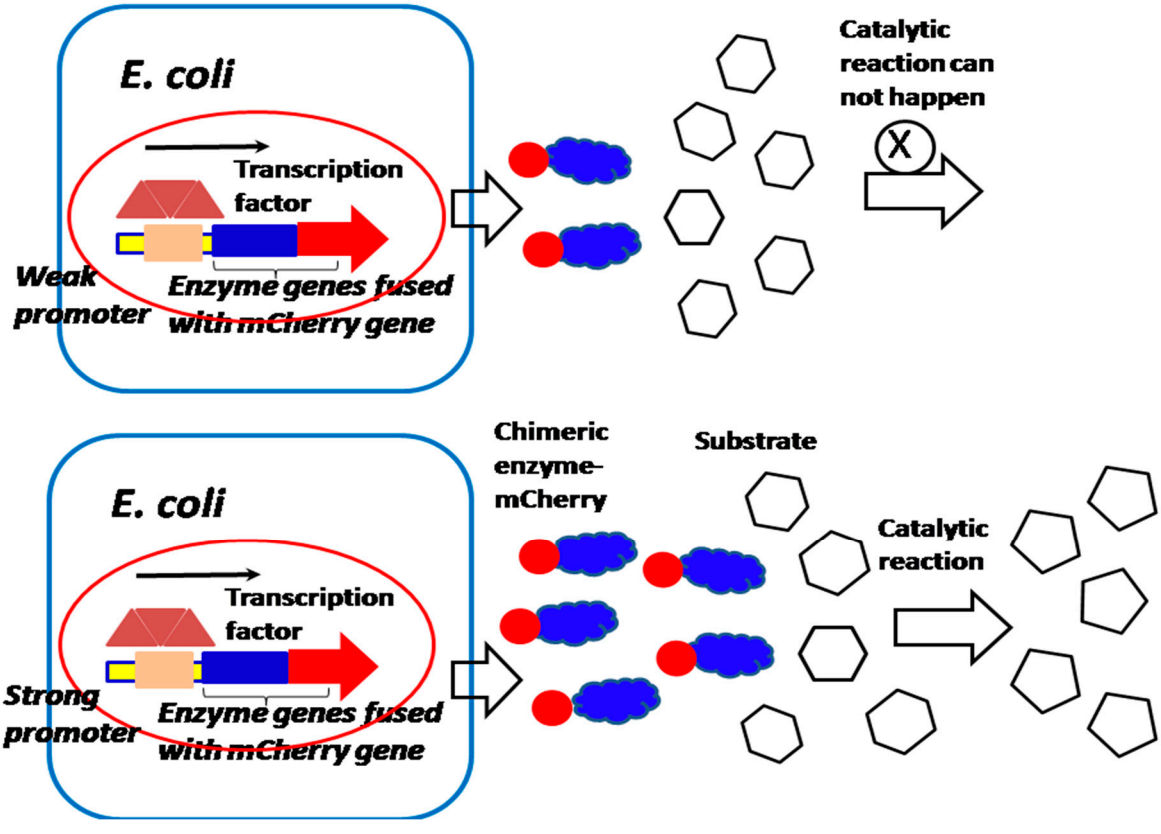

Figure 6. Performance enhanced promoter is applied for studying the enzyme activity.

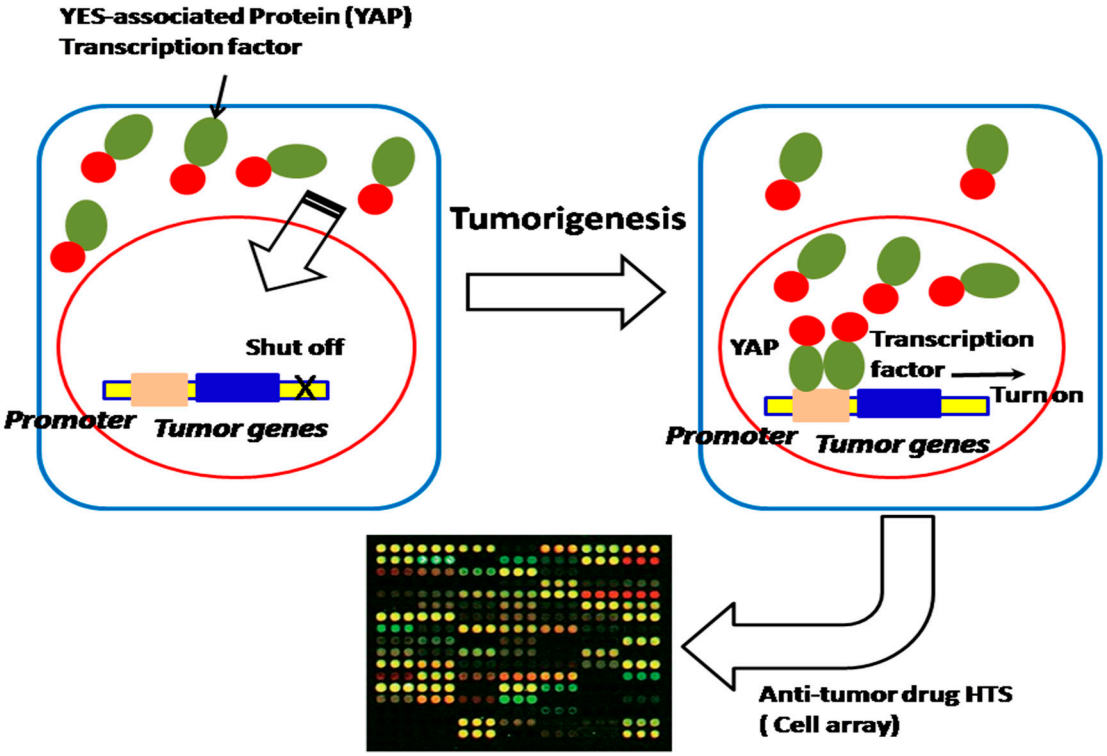

Figure 7. Performance enhanced promoter is applied for construction of HTS platform for HTS anti-tumor drugs and studying nucleocytoplasmic shuttling regulating factor YAP's nucleocytoplasmic localization and tumorigenesis, which control tumor growth and invasion.

2.3. Utilization of promoter for construction of sensitive bio-sensor for probing density of bacteria.

Due to the strong luminescent properties of immunofluorescent protein, we could integrate both low and high bacteria density responsive promoters into genetic regulatory circuit for probing the cell density[24,25](**Figure 8**). This bio-sensor could be in responses to AHL molecule and probe the growth density of bacteria which provide the instruction for how to balance the cell growth density to improve the valuable product production (**Figure 9**).

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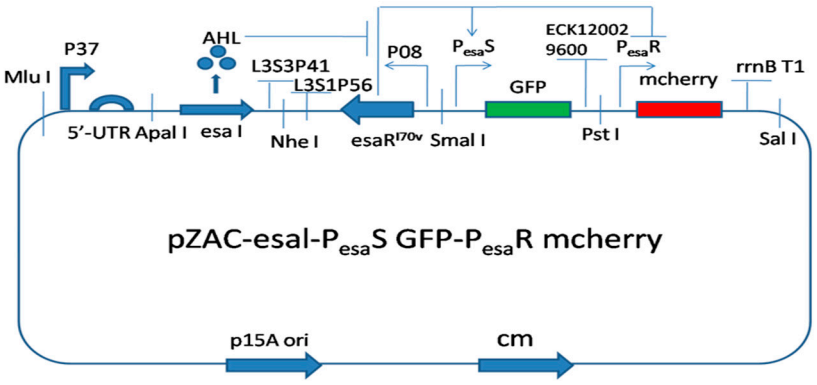

Figure 8. Both red and green fluorescent protein (*mCherry* and *GFP* gene) probe the density of bacteria.

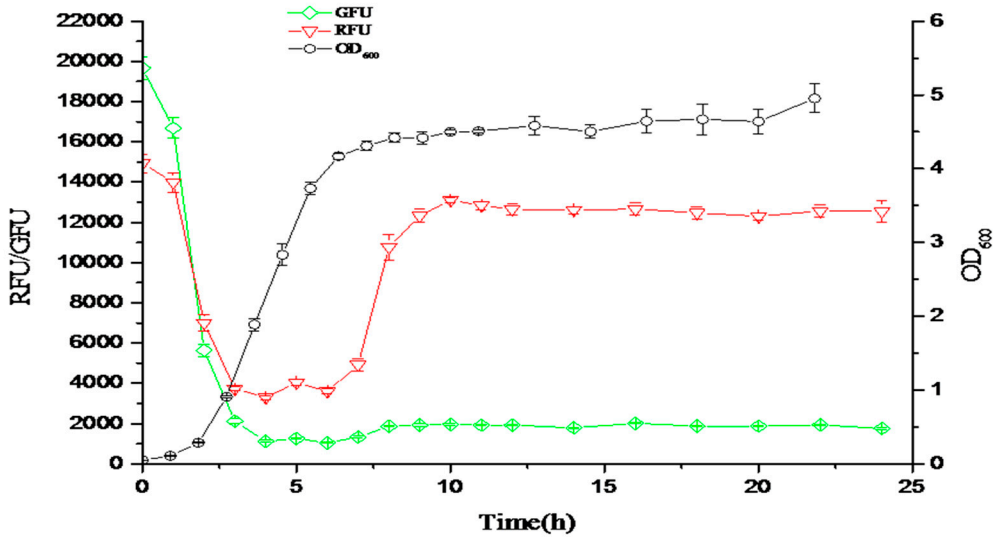

Figure 9. The responsive curves of fluorescent ratio (OD_{535}/OD_{600}) in responses to the density of bacteria.
Blue curve is the blue fluorescent ratio (OD_{535}/OD_{600}) in responsive to the growth of bacteria.
Red curve is the red fluorescent ratio (OD_{535}/OD_{600}) in responsive to the growth of bacteria.
Black curve is the cell density of bacteria (OD_{600}).

2.4. Mn^{2+} and Mg^{2+} added random Error-prone PCR mutagenesis approach for construction of the bio-sensor "library" that is regulated by inhibitory protein.

In contrary, resveratrol and shikimic acid synthesis is inhibited by ttgR and hucR inhibitory protein which is in responsive to resveratrol and shikimic acid[26,27]. In most time, the expression of ttgR or hucR constitutive genes is

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repressed by inhibitory protein without binding resveratrol or shikimic acid, however, the gene expression will be initiated in the condition of the resveratrol or shikimic acid binding which triggers the allosteric response that de-repress the inhibitory effect[26]. Because Mn^{2+} and Mg^{2+} added random Error-prone PCR mutagenesis approach can introduce random mutation sites into the DNA sequence, the properties of the gene with the promoter of inhibitory protein can be improved for resveratrol and shikimic acid binding, and the strong strength of the mutated gene with promoter could be screened from the "mutant library" (**Figure 10**). Therefore, random mutation of promoter by Mn^{2+} and Mg^{2+} added Error-prone PCR can increase promoter's sensitivity to inhibitory protein, which rapidly de-represses the inhibitory effect and promote the resveratrol and shikimic acid production.

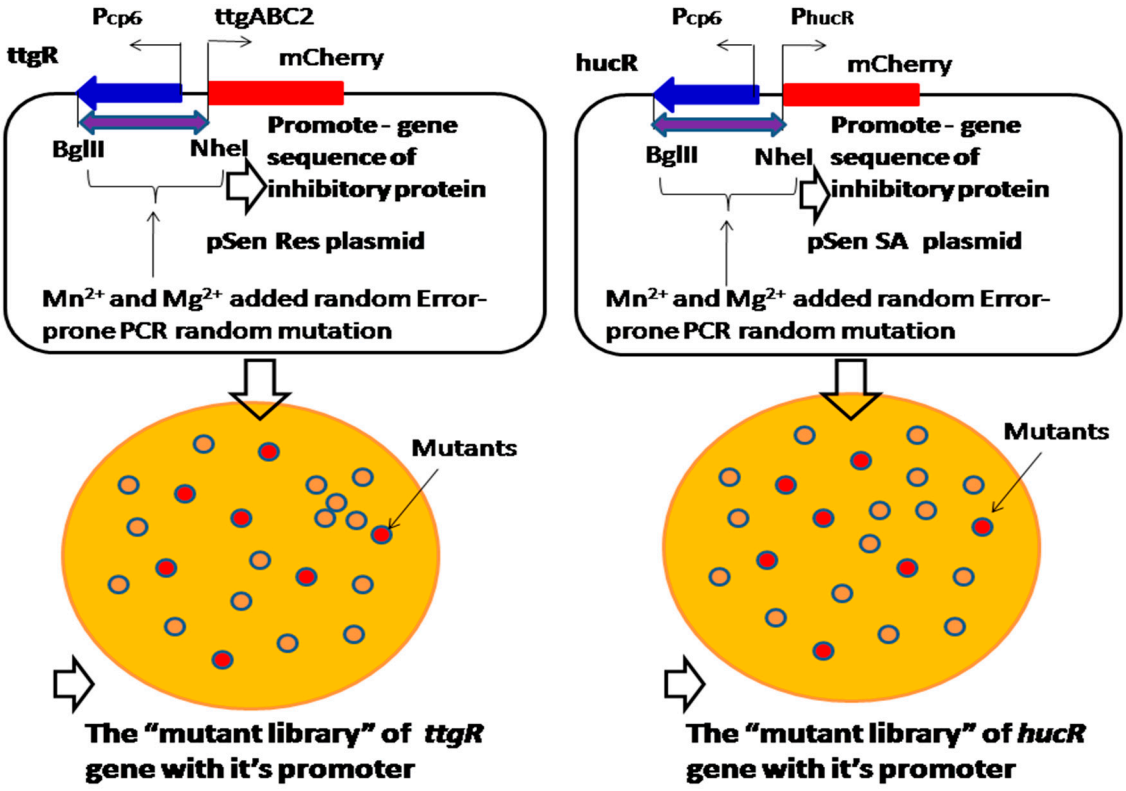

Figure 10. Mn^{2+} and Mg^{2+} added random Error-prone PCR mutagenesis approach for randomly mutating of the entire inhibitory protein gene with it's promoter sequence to construct the "mutant library" to screen the sensitivity of the bio-sensors.

2.5. Integration of both promoter-based bio-sensor and bacterium-host cell interactive mechanism for HTS meningitis drugs.

In our previous studies, we found that the membrane protein of *E. coli*, YojI can medicate the interaction of *E.*

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coli and human brain microvascular endothelial cells (HBMEC) by using both the human and *E. coli* proteome chips in conjunction with cell labeling techniques for discovery of microbial and host factors. We identified that YojI binds to the interferon-alpha receptor (IFNAR2) on the surface of human brain microvascular endothelial cells (HBMECs) and mediates E. coli adhesion to the host cells, and it is an important virulence factor for *E. coli* invasion of HBMEC. It is reported that YojI is also a chemical induced bio-sensor for regulating it's expression, a leucine-responsive regulatory protein, which Lrp controls the expression of YojI, which regulates exporting of toxin J25[28-30]. Therefore, the strong promoter can be indentified and cloned before chimeric gene *YojI-mCherry* gene to highly initiate the gene expression to strongly incorporate YojI-mCherry chimeric protein onto the cell surface. When the recombinant *E. coli* infect HBMEC, the strong promoter initiated the chimeric protein YojI-mCherry expression will amplify the invasion signaling of *E. coli* in HBMEC. As a result, the strength of the promoter of *YojI* gene is correlated with invasion efficiency of *E. coli*. Bacterium-host cell interactive mechanism integrated with high performance promoter can be effectively utilized for HTS screening meningitis drugs (**Figure 11**).

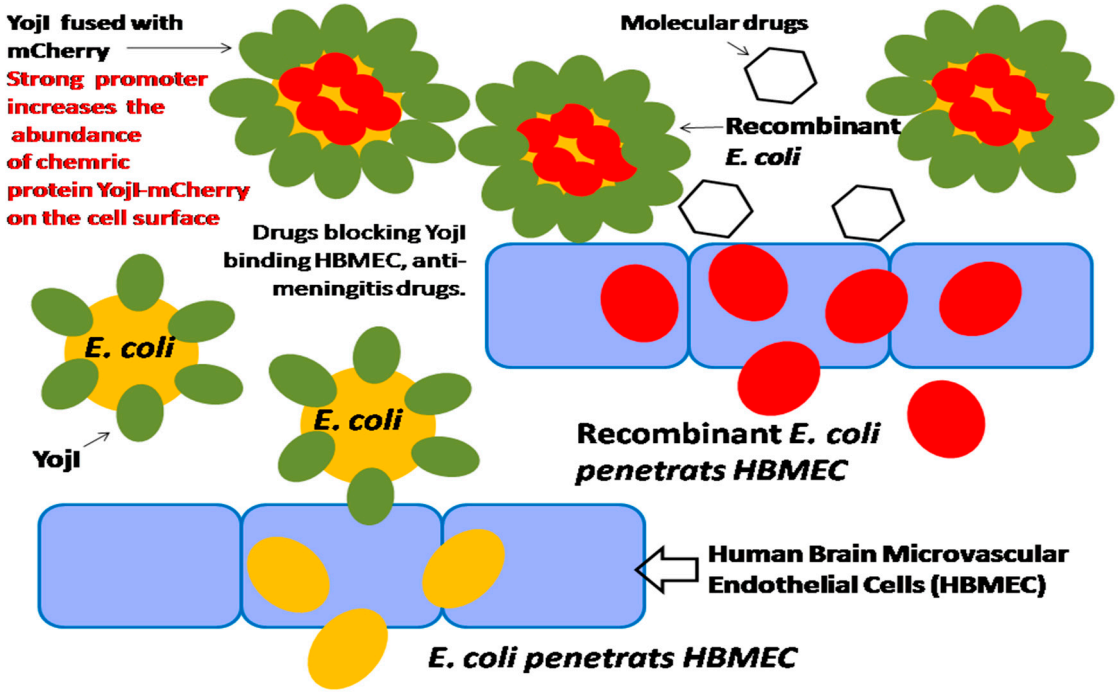

Figure 11. Integration of promoter-based bio-sensor and bacterium-host cell interactive mechanism for HTS meningitis drugs.

2.6. Construction of promoter-engineered genetic circuit for detection of intermediate toxic compound to improve the synthesis of value-added medicinal product yield.

The promoters to regulate pathway expression in responses to the intermediates can create a link between the cell's metabolic state and the expression of the metabolic pathway. It is reported that the coupling of synthetic promoters

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and metabolic engineering can improve the production of valuable chemicals. We have identified native promoters that respond to L-Ornithine and putrescine in our previous studies**.** On the one hand, once the L-Ornithine binding the transcription factors, it will trigger the allosteric response to active the expression of L-ornithine carboxylase which facilitate the rapid conversion and synthesis of L-ornithine from L-ornithine to putrescine. This is "push" regulatory mode of L-Ornithine that promotes the synthesis of putrescine. On the other hand, excessive accumulation of putrescine will bind it's promoter to active the expression of N-acetyl glutamic acid synthetase system ArgCJBD[31,32]. ArgCJBD will promote the conversion of glutamic acid to synthesis of L-ornithine, supplement the consumption of L-ornithine which increases the intracellular level of L-ornithine, and further promote the transformation to putrescine[15,31]. This is the remote "pull" regulatory mode of synthesis of putrescine. We expect to integrate both "push" and "pull" bio-sensors into a integrated genetic circuit to dynamically regulate synthesis of putrescine in *Corynebacterium glutamicum*. This "push" and "pull" regulatory mode will continuously promote the conversion and synthesis of from L- ornithine to putrescine, due to the ArgCJBD route can supplement the consumption of L-ornithine, which push forward the conversion of L-ornithine to putrescine (**Figure 12**). With the mentioned above approaches, we could synthesize the promoters to increase their responses strength and apply above mentioned Mn^{2+} and Mg^{2+} added random Error-prone PCR mutagenesis approach to further enhance the promoter's performance to greatly improve the synthesis of putrescine.

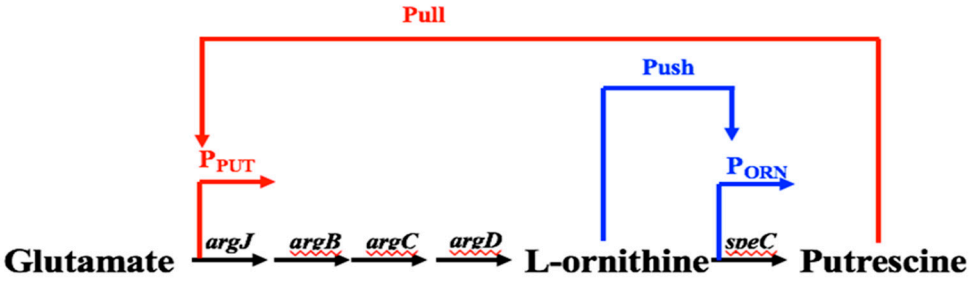

Figure 12. Construction of dynamic genetic circuit in "push" and "pull" mode to promote conversion of L-ornithine to putrescine.

3. Discussion and Conclusion

In this paper, a range of approaches have been proposed for engineering promoter-gene components for

construction of sensitive bio-sensors. Because the fluorescence intensity of *mCherry* and *EGFP* protein expression are correlated with the promoter's strength, the promoter-gene component can be engineered for construction of bio-sensors with human and bacteria arrays for diverse applications, such as HTS screening of transcription factor-acting and anti-tumor drugs, increase the abundance of membrane protein and enzymes to study their activities, observation of movement and localization of nucleocytoplasmic shuttling factor for tumorigenesis and oncology mechanism investigation, application of both mCherry and EGFP protein for probing the density of bacteria to improve synthesis of high valuable product, and construction of dynamic genetic circuit for monitoring of intermediate toxic compound, which can be applied in the food and environmental toxic substance testing. In novelty, the YojI regulatory bio-sensor combined with *E. coli* invasion mechanism of HBMEC can be utilized for HTS screening meningitis drugs and discovery of other infection disease therapy[33-35]. Although there are many applications of bio-senor proposed in this paper in human health and environment, the properties of promoter-gene component included the sensitivity and specificity of the bio-sensor are not too hard to be handled, because Mn^{2+} and Mg^{2+} added random Error-prone PCR mutagenesis approach could introduce random mutation sites to the promoter-gene component and corresponding fragment to construct the "mutant library", and the mutants with improved properties can be screened by this "library". The promoter strength can be further improved by easily synthesizing them for increasing the responsive strength of bio-sensors. As these proposed method develops, the new enzyme activity with a designed bio-sensor can be discovered. This novel gene switch allow us to stringently control virulent protein expression and study more new enzyme function[26,31].

In a conclusion, promoter-gene component engineered strategy have the potential to revolutionized recent biotechnological development and allow the rapid engineering of required circuits to increase the particular value-added products in bio-medicinal field.

Author Contributions: Y.Z.F wrote the whole manuscript. J.Z.L., B.C.W., Z.Z.X., X.L.J., Z.L.,Y.P.S provided the technical support for this manuscript.

Acknowledgements: We thank Y.Z.F' wife X.N.J. provided the language editing and advice for this manuscript.

Conflicts of Interests: The authors declare no conflict of interest.

Peer-reviewed version available at Sensors 2018, 18, 2823; doi:10.3390/s18092823

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