Cloning and Expression of Deep Red Fluorescent Protein Gene

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Abstract: The deep red fluorescent protein gene DsRed2 was extracted from the original plasmid to form a prokaryotic expression vector, and the clone and expression test of the deep red fluorescent protein gene was carried out. The test results showed that the fluorescent protein gene could be cloned and cultured in liquid culture medium at 37℃ for 1 to 5 hours, and it had strong chromogenic ability. Deep red fluorescent protein can easily distinguish bacterial colonies between positive and false positive and help cell localization.

Keywords: Deep red fluorescent protein; Protein gene cloning; Protein gene expression

1. Introduction

Deep RFP (Red Fluorescent Protein) is a kind of biological protein, which is extracted from the Discosomasp sp, has light-emitting properties. The gene code of the deep RFP is DsRed, and the red fluorescence emitted is highly penetrable and has a very long duration of penetration. Compared with GFP (Green Fluorescent Protein) and YFP (Yellow Fluorescent Protein), the fluorescence intensity of deep RFP is worse, but the mutation is higher, anti-bleaching ability is better; it is a new type of visual reality nuclear expression genes that can help cell localization. The disadvantage of deep RFP is that it needs to use ultraviolet light and other special light sources to stimulate so as to form stable structure of homologous tetramers; if there is no special light irradiation, deep RFP may form insoluble multimers. The expression characteristics and influencing factors of the gene were studied to analyze the whole cloning and expression process, and to provide theoretical and experimental basis for its gene resources [1].

2. Materials and Methods

2.1. Materials

The selected gene strain was E.coli (provided by Peking University Life College), and the selected prokaryotic expression gene strain was DE3 (provided by the Science Laboratory of the Chinese Academy of Sciences) [2].
The TMPI vector was used as the vector of the target gene strain, and PMDI was used as the vector for the prokaryotic expression gene strain. The test equipments used include PCR kit, IED restriction endonuclease, HIE restriction endonuclease, Tis ligase (provided by American biotechnology company pwecad), PCR product and plasmid recovery box (provided by British Biological Co., Ltd. SEM), the important reagents in the test are imported.

2.2. Test methods

According to the gene expression sequence of DsRed2 design and compose primer. The restriction endonuclease endpoint was designed at the upstream of the strain and labeled as Bam1; the ligase was designed at the downstream of the strain and labeled as Asc1. The strains were digested with different restriction endonuclease, and the requirements for the construction of prokaryotic and eukaryotic expression vectors were met. The upstream sequence is recorded as follows: TAA CIA ATA TCC GAA TGG TAC CTC; the downstream sequence is recorded as follows: CGA CCC TAG TCA AGC TCC ACA GTT. The sequence of the upstream and downstream sequences is between 600 bp to 750 bp. After designing and composing primers, the target gene DsRed2 was isolated by PCR cloning method. The target gene PCR was amplified in Promega amplifiers. It was divided into five stages: pre-denaturation, denaturation, renaturation, extension and circulation. The reaction temperature of predenaturation is 90℃, the reaction time is 3min to 5min, the reaction temperature of denaturation is 92℃, the reaction time is 75s, the reaction temperature of renaturation is 60℃, the reaction time is 7min, the reaction temperature of extension is 40℃, the reaction time is 45s, the reaction temperature of circulation is 72℃, and the cycle time is 52 [3].
After completion of the above procedures, the target gene DsRed2 was cloned and the clonal results were identified. The target DNA fragment was amplified by PCR and recovered and purified on a Bio screening machine, and then ligated with PMD vector to obtain the recombinant gene PMD-DsRed2. The recombinant gene PMD-DsRed2 was incorporated into DE3 perceptual gene cells by heat shock method. Monoclonal was picked up on the solid culture medium containing ampicillin and after cloning it was transferred to liquid culture medium for

expanding culture. After a period of time, the cultured product was removed, the inside of the plasmid was purified, the internal sequence was identified by a PCR machine, and then the internal software was analyzed by computer software.

After obtaining the correct identification result, PMD-DsRed2 was digested to obtain a DNA sequence. And it was ligated by TWDNA ligase to obtain prokaryotic expression vector. The obtained vector was placed in selective culture medium and propagated, and the plasmid was extracted to determine the target DNA sequence and its expression in Escherichia coli [4]. The plasmid containing DNA was placed in 100 ml of liquid LB medium at a ratio of 1: 100 and processed by shocks with 180 rpm per minute. After 5 hours, 0.1 ml of TPG solution was added for 48 hours, and the culture temperature was kept at 37℃. After the induction culture was completed, the cells were pulverized by ultrasonic irradiation and transferred to a hollow tube. After 15 minutes of centrifugation, the upper and lower layers of the solution were obtained. PBS buffer was added to the solution to obtain a precipitate and analyzed by electrophoresis sequence, and display the results in the computer.

3. Results and Analysis

The expression image of the deep RFP obtained by the above selection of the reagents and experimental methods is shown in Figure 1:

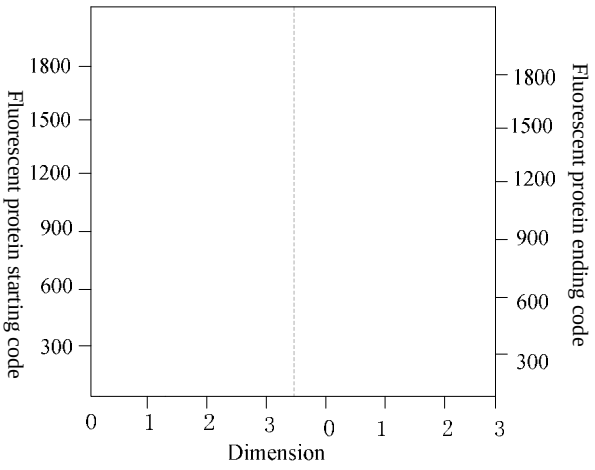

Figure 1. Expression Image of Deep RFP

Analyzing the sequencing results of Figure 1, the target DNA sequence length of plasmid DsRed2 separation is 680pb, amino acid protein is 225, open reading frame is 675. There was one base difference between the fluorescent protein starting code and the fluorescent protein ending code, and there was a high agreement between the two sequences, and the mutation way was synonymous. The constructed expression vector has a continuous coding in the DNA sequence, and there is no coding mutation [5].

The color characteristics of RFP can be reflected in the GEX4T1 color plate. Cultured in liquid culture medium for 24 hours, then selected the plasmid representative of red colony and transferred to the coating for 48 hour's culturing, the culture temperature maintained at 37 ℃, the results obtained as shown in Figure 2:

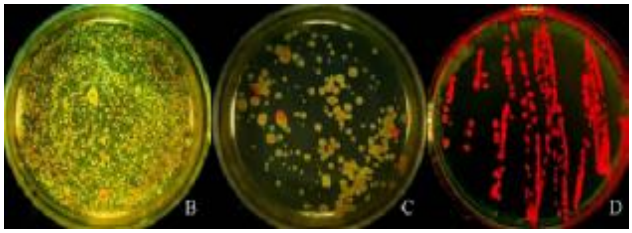

Figure 2. Expression Characteristics of Deep Protein Gene

Results in Figure 2 show that the target gene can be expressed in a common strain and can also be expressed in a cloned strain. Under the induction of natural light, red (positive) and white / non-red (false positive) colonies can be distinguished by observation. After the transformation, colony has many species, fast breeding speed and color significantly.

4 . Conclusions

Deep RFP can help cell localization and can be localized in natural light. The deep EFP gene needs to be propagated in liquid culture medium at 37℃ for 48 hours, and the strains were digested with different restriction endonuclease to form different sequences and complete cloning. Under the inducement of ultraviolet laser, the target gene will express a bright red, which is a visual reporter gene with great potential.

References

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