2014

PCR Amplification of the Green Fluorescence Protein Gene for Sub-Cloning

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Abstract

PCR was used to amplify the green fluorescence protein (GFP) gene present on the pGLO plasmid with custom designed PCR primers that included: 1) a leader sequence, 2) a restriction endonuclease recognition sequences for the cloning site, and 3) a partial sequence complimentary to the GFP gene. The GFP gene amplicon was ligated to digested pUC18. The recombinant pUC18 was transformed using chemically competent E. coli DH5α. A blue/white screening method showed the presence of recombinant clones. The presence of GFP on recombinant pUC18 was confirmed through PCR and second cycle of cloning.

Introduction

- *The GFP gene from Aequorea victoria* (jellyfish) is frequently used as a gene marker in molecular techniques.
- *The GFP gene was amplified from the Bio-Rad pGLO plasmid using PCR and purified.*
- *A recombinant pUC18 plasmid with GFP gene was cloned in E. coli DH5α cells and recovered.*
- *Cloned recombinant pUC18 plasmid was re-isolated and used for a second transformation.*
- *The recombinant clones were selected by blue-white screening.*

Methods

The GFP gene was amplified in a thermo cycler (30 cycles) using puReTaq Ready-To-Go PCR beads. The Bio-Rad pGLO plasmid shown in Figure 1 was the source of GFP gene. The Thermo Scientific GeneJET PCR Purification kit was used to purify amplified DNA. New England Biolabs (NEB) restriction endonucleases EcoRI and PstI were use for the restriction digestion of purified GFP and pUC18. The recombinant pUC18 in Figure 2 was obtained from ligating GFP amplicon and pUC18 with T4 DNA ligase (NEB) at 16°C for 16 hours. Gel electrophoresis on 0.8% agarose gel was used to identify and measure DNA concentrations and lengths using an NEB 1kb DNA ladder.

Figure 1. pGLO plasmid

Figure 2. Recombinant pUC18 plasmid

The PCR primers (1) shown in Table 3 were used for GFP amplification and cloning and obtained from Integrated DNA Technologies.

Table 1. GFP PCR Primers

Primer	Primer sequence	Restriction sequence	Complementary sequence	Leader Sequence
GFP – f2	ACAGCCGAATTCATGGCTAGCAAAGGAGAA	GAATTC	ATGGCTAGCAAAGGAGAA	ACAGCC
GFP – r2	GGATGTCTGCAGTTATTTGTGAGAGCTCATCCAT	CTGCAG	TTATTTGTGAGCTCATCCAT	GGCTGT

The Hanahan (2) method was used to prepare competent E. coli DH5α cells. A blue-white screen was used to isolate white recombinant from blue non-recombinant colonies grown on Ampicillin, IPTG, X-gal selective Luria agar plates. White colonies were indicative of DNA ligation disrupting the α-complementation of the β-galactosidase gene.

Results

Figure 3 shows digested pUC18 (1 – 3), 1kb DNA ladder (4 &9), digested GFP (5 & 6), undigested GFP (7 & 8).

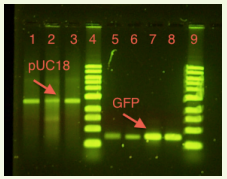

Figure 3. Digested pUC18 & GFP

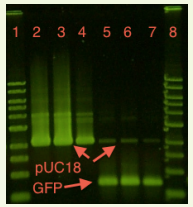

Figure 4. Cloned recombinant pUC18 & GFP

Figure 4 shows 1kb DNA ladder (1 & 8), recombinant pUC18 (2 – 4), PCR amplified GFP from cloned recombinant pUC18 (5 – 7).

The size of cloned recombinant pUC18 shown in Fig. 4 (2 - 4) was 2 – 3 kb. This DNA was from white colonies transformed with cloned recombinant pUC18 recovered from the first transformation. The size of the PCR GFP amplicon from cloned recombinant pUC18 shown in Fig. 4 (5 – 7) was 0.5 – 1 kb. The white colonies didn't fluoresce under 365 nm UV light. However, pGLO transformed white colonies shown in Fig. 5 did fluoresce under 365 nm UV light.

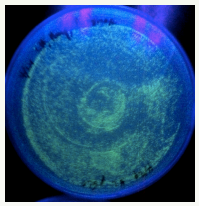

Figure 5. E. coli transformed with pGLO

Conclusion

The recombinant pUC18 recovered from white colonies was in the expected 2 – 3 kb size range and was shown by PCR to contain the GFP insert. The white colonies didn't fluoresce. Potential reasons for absence of fluorescence include: 1) The GFP insert contained a mutation(s), since PCR Taq polymers lacks proof reading function. 2) The GFP insert in the lacZα gene created a non-fluorescing hybrid protein as shown in Figure 6.

Figure 6. Recombinant pUC18

3) The inserted GFP gene wasn't in frame with lacZα gene, which corrupted the transcription/ translation process.

Further investigation is needed to determine why the recombinant pUC18 plasmid didn't express fluorescing GFP protein. This would include sequencing the GFP PCR product and cloning GFP in an expression vector such as pET.

References

1. *Addgene: A Better Way to Share Plasmids. Web. 8 May 2014. <https://www.addgene.org/plasmid_protocols/PCR_cloning/>.*

2. *Green, Michael R., and Joseph Sambrook. "Chapter 3: Cloning and Transformation with Plasmid Vectors." Molecular cloning : a laboratory manual. 4th ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 2012. 157-260.*