www.sospublication.co.in

Journal of Advanced Laboratory Research in Biology

We- together to save yourself society

e-ISSN 0976-7614

Volume 3, Issue 3, July 2012

Research Article

# Construction of Novel Phytochelatins by Overlap Oligonucleotides

Behnaz Saffar*, Mohsen Mobini and Azadeh Mohamadi

*Genetic Department, Faculty of Science and Research Institute of Biotechnology, Shahrkord University, Shahrkord, Iran.*

**Abstract:** Synthetic phytochelatins are protein analogs of phytochelatin with similar heavy metal binding affinities that can be easily produced from a synthetic DNA template. We design synthetic phytochelatin [(Glu-Cys)n Gly] linked to hexahistidine by viral linker peptide and then followed by gene synthesis and cloning of it. Then peptide coding gene (synthetic phytochelatin with linker and hexahistidine) was designed exactly and constructed with step by step methods by overlapping oligonucleotides using T4 DNA Ligase. Finally, synthesized gene amplified by PCR, cloned in pTZ57R/T and transformed to *Escherichia coli* (DH5α). The results of sequencing show that some types of synthetic phytochelatin (EC4, EC12, and EC20) with linker and hexahistidine were constructed and cloned in vector.

**Keywords:** Design of oligonucleotide, Hexahistidine, Synthetic phytochelatin.

## 1. Introduction

Heavy metals pollution is one of the most important environmental problems today that has an adverse effect on human health and ecosystems. Conventional technologies such as precipitation-filtration, ion exchange, and reverse osmosis, oxidation-reduction and membrane separation are often inadequate to reduce metal concentration to acceptable regulatory standards [1]-[2].

Metallothioneins and phytochelatins are peptides that effectively bind a wide range of heavy metals [3]-[4]. One of the best characterized heavy metal binding ligand in plant cells is phytochelatins. Phytochelatins (PCs) are short cysteine-rich peptides with the general structure (γ-glu-cys)n-Gly, where n=2-11. Isolation and purification of PCs from plants or organisms is difficult. Synthetic phytochelatins (ECs) are analogs of PCs that have an α peptide bond instead of the γ peptide bond. Unlike PCs, ECs can be produced using the cell ribosomal machinery [5]-[6].

Additionally, ECs of different chain length even higher than that are found in natural plants can be produced to provide peptides with different metal binding capacities [7]. In this study, we have designed novel phytochelatins ECn ≥ 4 linked to hexahistidine by helical linker. The hexahistidine tail can be used for detection and purification of protein and develop high affinity adsorption.

## 2. Materials and Methods

The synthetic gene encoding for (Glu-Cys)n Gly linked to hexahistidine were designed using 8 oligonucleotides ranging from 19-48 nucleotides in length (Table 1). This linker sequence "RGRWMARLARM" was taken from the linker library (www.ibi.vu.nl) [8]-[9]. Molecular dynamics simulation will ensure the linker properly selected [10]-[11].

*Corresponding author:
E-mail: saffar_b@sci.sku.ac.ir; Phone: 983814424419; Fax: 98381442419.

**Table 1. The oligonucleotides for construction of synthetic phytochelatin.**

| NAME OF OLIGO | SEQUENCE |
|---|---|
| O1 | TTTGGATCCATATGGAATGTGAATGTGAATGTGAATGT |
| O2 | ACATTCCATATGGATCCAAA |
| O3 | GAATGTGAGTGCGAGTGCGAGTGCGAGTGCGAATGTGA ATGTGAATGT |
| O4 | GCACTCGCACTCGCACTCGCACTCACATTCACATTCACA TTCACATTC |
| O5 | GGTCGTGGCCGTTGGATGGCGCGCCTGGCGCGCATGCA TCATCAC |
| O6 | CGCCAGGCGCGCCATCCAACGGCCACGACCACATTCAC ATTCACATTC |
| O7 | CATCATCACTAAGCTTAAA |
| O8 | TTTAAGCTTAGTGATGATGGTGATGATGCATGCG |

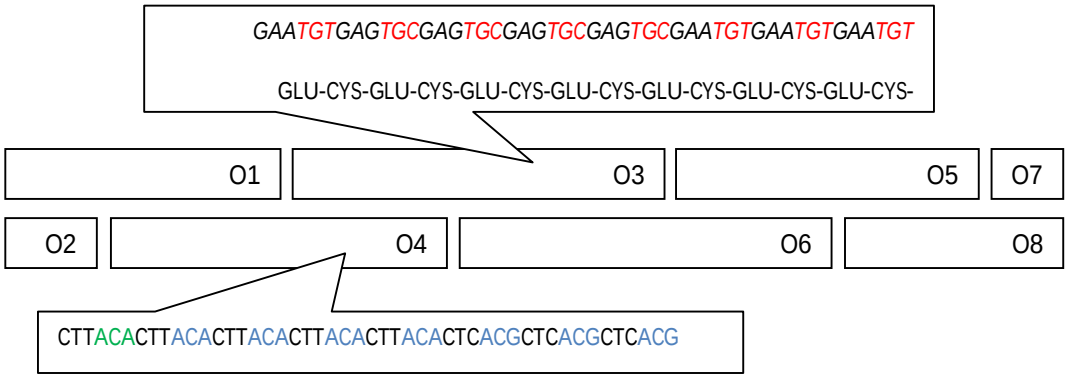


**Fig. 1. Schematic diagram of designing oligos that codes (Glu-Cys)n Gly linked to hexahistidine.**

Oligo design was difficult because these oligonucleotides have a repetitive sequence of glutamic acid and cysteine and overlap sequences can be paired together in another unwanted frame. Therefore, Oligos were designed with lowest frame indisposition. All oligonucleotides were checked by the Gene runner program for their GC content, hairpin loops, bulge loops and oligo dimmers. In addition, oligonucleotides O3 and O4 can be repeated in the construction of (Glu-Cys)n Glyn $\geq 4$ (Fig. 1). Oligos were purchased from TAG Copenhagen A/S with RP-column purification and MALDI-TOF quality control. The solution of all the oligonucleotides was made with 100ìM concentrated by adding sterile $ddH_2O$. In the first, a pair of oligonucleotides O1 with O2, O5 with O6 and O7 with O8 were mixed and annealed. In the second step, O3 and O4 oligos add to mix of O1 and O2 separately and annealed. Then, mix of O5 and O6 and O7 and O8 add to assembly mix. Assembly mix solution was made in which the concentration of each oligonucleotide was 1µM. By dilution of this mix, we could obtain the desired concentration of each oligonucleotide in the ligation reaction mix. In the ligation reaction mix, a solution was made with T4 ligation buffer and T4 ligase in a final volume of 10µl. The mixture was incubated at 22°C overnight [12]-[13]. The following PCR was carried out in 25µl reaction mixture containing 1µl ligation mixture, 0.1µM of the first and last oligos as primer, 2.5µl PCR buffers 10X, 1.5µl $MgCl_2$ (50mM), 1µl dNTP (10mM) and 1U *Taq* polymerase (Cinnagen).

The PCR reaction was carried out in 30 cycles of:

(i) Denaturing at 94°C for 30s,
(ii) Annealing at 55°C for 30s and
(iii) Extension at 72°C for 1min.

Duration of final extension was 5 min. The PCR product of reactions was loaded on 1% agarose gel and then cloned into the T/A cloning vector and verified by restriction endonuclease mapping of their plasmids. Accuracy of the sequence was checked by sequencing (Macrogen Company).

## 3. Results and Discussion

The result of sequencing shows that the Synthetic gene coding for EC4, EC12, EC20 were synthesized, linked to hexahistidine by helical linker. The PCR product obtained after amplification of synthetic phytochelatin is illustrated in Fig. 2.

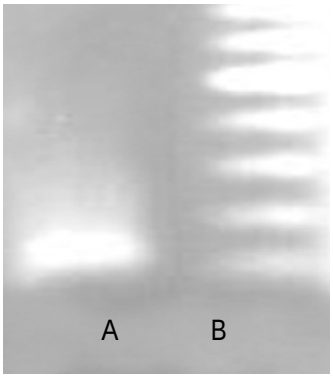


**Fig. 2. Agarose gel electrophoresis of the PCR product, lane B is a molecular weight marker 100bp ladder; lane A is the PCR product of synthetic phytochelatin.**

Various methods have been proposed over the last three decades for *in vitro* gene synthesis, including oligonucleotide ligation, and PCR. Among them, assembly PCR and ligase chain reaction coupled to PCR are most suitable for gene synthesis [14]-[15]. The genes coding ECn have repetitive sequence, so, we change method of oligonucleotide ligation to produce ECn $\geq$ 4. In this method, oligonucleotide adds to reaction step by step in construction of correct frames. In addition, we economize in the use of oligonucleotide in construction of ECn.

Weon Bea demonstrates *E. coli* strain that displays synthetic phytochelatins ranging from 8-20 cysteines (EC8, EC11 and EC20) confer metal binding capability on the host cells and the resulting novel bio-adsorbents accumulate a substantially higher amount of cadmium than the wild-type cells [16]. So we decided that change to conform of synthetic Phytochelatins with linked to hexahistidine for developing high-affinity bio-adsorbents suitable for heavy metal removal because polyhistidine peptide was used as a model insert for development of a heavy metal uptake system for environmental application [17].

In addition, hexahistidine can be used for purification and detection of protein. The reason for choosing this linker was helical and rigid structure of it. Of course, because of the addition of Gly at the end of this linker, it is not very rigid. Since hexahistidine tail may interfere with ECn sequence a helical linker was included between ECn sequence and hexahistidine tail. Helical linkers are thought to act as rigid spacers to prevent non-reactive interaction between domains [11]. The rigidity of linker helps it to separate two parts of ECn sequence and hexahistidine tail.

## 4. Conclusion

In summary, we synthesized series of genes that encode peptides from EC4 to EC20 linked to hexahistidine by use of the overlap oligonucleotide method. These genes were cloned into cloning vector and accuracy of the sequencing was checked. In the future, we have to clone these genes in various expression vectors for production of protein and estimation of heavy metal adsorption.

## Acknowledgment

The authors gratefully acknowledge the Biotechnology Institute of Shahrekord University and financial support from the Research Council of the Shahrekord University is gratefully acknowledged.

## References

[1]. Bae, W., Mulchandani, A., Chen, W. (2002). Cell surface display of synthetic phytochelatins using ice nucleation protein for enhanced heavy metal bioaccumulation. *Journal of Inorganic Biochemistry*, 88: 223-227.

[2]. Bontidean, I., Ahlqvist, J., Mulchandani, A., Chen, W., Bae, W., Mehra, R.K., Mortari, A., Csöregi, E. (2003). Novel synthetic Phytochelatin-based capacitive biosensor for heavy metal ion detection. *Biosensors and Bioelectronics*, 18:547-553.

[3]. Cobbett C.S. (2000). Phytochelatins and their roles in heavy metal detoxification. *Plant Physiology*, 123: 825-832.

[4]. Bae, W., Mehra, R.K., Mulchandani, A. and Chen W. (2001). Genetic engineering of *E. coli* for enhanced uptake and bioaccumulation of mercury. *Applied and Environmental Microbiology*, 67: 5335-5338.

[5]. Bae, W. and Mehra, R.K. (1997). Metal-binding characteristics of a phytochelatin analog $(Glu\text{-}Cys)_2Gly$. *Journal of Inorganic Biochemistry*, 68: 201-210.

[6]. W. Bae, W. Chen, A. Mulchandani, and R.K. Mehra (2000). Heavy metal removal using bacteria displaying synthetic phytochelatins. *Environmental Chemistry*, 40: 793-794.

[7]. Cobbett, C., Goldsbrough, P. (2002). Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. *Annu. Rev. Plant Biol.*, 53: 159-82.

[8]. Xue, F., Gu, Z., Feng, J.A. (2004). LINKER: a web server to generate peptide sequences with extended conformation. *Nucleic acids Research*, 32: W562-5.

[9]. Zhang, J., Yun, J., Shang, Z., Zhang, X., Pan, B. (2009). Design and optimization of a linker for fusion protein construction. *Progress in Natural Science*, 19: 1197-1200.

[10]. George, R.A. and Heringa, J. (2002). An analysis of protein domain linkers: their classification and role in protein folding. *Protein engineering*, 15: 871-9.

[11]. Mahnam, K., Saffar, B., Moboni, M., Mohamadi, A. Design and construction of a novel metal

binding peptide for sequestering of heavy metals by molecular dynamics simulation. Unpublished.

[12]. Borodina, T.A., Lehrach, H., Soldatov, A.V. (2003). Ligation-Based synthesis of oligonucleotides with block structure. *Anal. Biochem.*, 318: 309–313.

[13]. Rouillard, J., Lee, W., Truan, G., Gao, X., Zhou, X., Gulari, E. (2004). Gene2oligo: oligonucleotide design for *in vitro* gene synthesis. *Nucleic Acids Research*, 32: W176-180.

[14]. Hoover, D.M., Lubkowski, J. (2002). DNAWorks: an automated method for designing oligonucleotides for PCR-based gene synthesis. *Nucleic Acids Res.*, 30: e34.

[15]. Au, L.C., Yang, F.Y., Yang, W.J., Lo, S.H., Kao, C.F. (1998). Gene synthesis by an LCR-based approach: high-level production of leptin-L54 using synthetic gene in *Escherichia coli*. *Biochem. Biophys. Res. Commun.*, 248: 200-203.

[16]. Bae, W., Chen, W., Mulchandani, A., Mehra, R.K. (2000). Enhanced bioaccumulation of heavy metals by bacterial cells displaying synthetic phytochelatins. *Biotechnology and Bioengineering*, 70: 518-524.

[17]. B. Saffar, B. Yakhchali, M. Arbabi (2007). Bioaccumulation of heavy metal ions by *E. coli* cells displaying hexahistidine peptide using a novel display system based on CS3 pili. *Current Microbiolog*., 55: 273-277.