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Optimization the Production of the Sticky Ends of Human *CCRL1* Gene

[1]Mohammad Karimian, [1]Abolghasem Esmaeili,
[2]Mohadese Behjati and [2]Mohammad Hashemi
[1]Department of Biology, Cell, Molecular and Developmental Biology Division,
School of Sciences, University of Isfahan, Isfahan, Iran
[2]School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract: CCRL1 encoded by gene belongs to the family of G-protein coupled receptors. This protein is a kind of C-C chemokine receptors which has a basic role in evolutionary function of heart identified chemokines. The aim of this study is to improve the creation of sticky ends at *CCRL1* gene by T-vector. In this study, *CCRL1* gene was amplified by PCR. Restriction sites of BamHI and NotI enzymes were introdused into the 5'end of the forward and reverse primers, respectively. Mentioned enzymes are in the endonuclease family group therefore can not create sticky ends at both sides of *CCRL1* gene to overcome this problem amplified fragment by Taq polymerase enzyme was inserted into T-vector. Then, recombinant vector was transformed in *E. coli*.

Key words: T-vector, white colony, blue colony, endonuclease, taq polymerase, Iran

INTRODUCTION

Seven trans-membrane receptors are a group of proteins with seven helical regions located in cell membrane (Khoja *et al*., 2000). Helical regions include 20-30 aminoacids that linked with three extracellular and intracellular loops.

These receptors have an extracellular amino-terminal and an intracellular carboxy-terminal. Since, these receptors adjoin to G-proteins, those are also named G-Protein Coupled Receptors (GPCR). These receptors only exist in eukaryotic cells (King *et al*., 2003). Several factors as neurotransmitters, peptides, ions, hormones and ligands are bound to these receptors and lead to signal transduction within these channels. Amino acids in helical regions of these receptors are highly conserved (Joost and Methner, 2002). The GPCRs are categorized to 100 sub-families according to sequence similarity and ligand structure and function.

Despite of sequence differences in GPCR's subgroups, all of them have same structure and signal transduction mechanisms.

Some of the used bioinformatics methods for classification of GPCRs are just based on the similarity of their amino acid sequence (Xiao *et al*., 2009; Qiu *et al*., 2009; Gu *et al*., 2010). In this study, we describe the optimization the creation of sticky ends at both sides of *CCRL1* gene by T-vector.

MATERIALS AND METHODS

RNA extraction and cDNA synthesis: Human spleen tissue was provided from accidental patients. The 30 mg of the spleen tissue was homogenized and RNA was extracted by RNeasy mini kit (QIAGEN). Extracted RNA was dissolved in distilled water. To ensure the integrity of extracted RNA, 2 μL of each sample was electrophoresed and then the Optical Density (OD) of each sample was read out at 260 nm. At next stage total cDNA was synthesized from extracted RNA by cDNA synthesis kit (Fermentas). To confidence the accuracy of cDNA synthesis, PCR reaction was performed on each sample for amplification of GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) House keeping gene.

Amplification of human *CCRL1* gene: In order to design specific primers, human *CCRL1* gene was deduced from NCBI (Accession number: AY221094.1) and analyzed with oligo6 software. The restriction sites of BamHI and NotI enzymes were designed at 5'end of forward and reverse primers, respectively.

The sequence of forward and reverse primers is 5'-AA AGGATCCGCTTTGGAACAGAACCAGTCA-3'and5'-GG GGCGGCCGCTTAAATGCTAAAAGTACTGGT-3', respectively (restriction sites are shown by italics). Primers were ordered from Metabion Company (Germany). To amplify the *CCRL1* gene, PCR reaction was performed

Corresponding Author: Mohadese Behjati, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

as follow: 0.3 μL of Smar Taq Polymerase (Stock: 5 U μL^{-1}), 0.5 μL dNTP (stock: 10 mM), 0.5 μL from each forward and reverse primers (stock: 100 pM), 0.75 μL $MgCl_2$ (stock: 50 mM), 2.5 μL 10X PCR buffer and 2 μL cDNA (1000 ng mL^{-1}). Final volume of 25 μL reached by addition of ddH_2O in the PCR mixture. (All PCR reagents were purchased from Fermentas). The reaction was done by thermocycler (Astec) as follow: Initial denaturation at 94°C for 5 min was followed by 30 repetitive cycles including denaturation temperature: 94°C for 1 min; annealing temperature: 56°C for 1 min, extension temperature: 72°C for 1 min. The amplified product was precipitated and dissolved in ddH_2O for further applications.

Insertion of amplified fragment into T-vector: The 1 μL of amplified human *CCRL1* gene was added to 10 μL from T-vector solution kit (Fermentase). For ligation purposes, this mixture was placed at 16°C for 12 h. Then, the mixture was transformed to *E. coli* cells. Transformed *E. coli* was cultured on media containing X-gal and IPTG. Recombinant vector was extracted from white colonies. Extracted vector was digested by BamHI and NotI enzymes according to the protocol.

RESULTS AND DISCUSSION

Amplification of CCRL1: As shown in Fig. 1a, the total spleen RNA, loaded on agarose gel, composed of 28, 18 sec and degraded RNA bands. The cDNA synthesis was approved by GAPDH amplification (Fig. 1b). Using the synthesized cDNA as template, *CCRL1* gene containing fragment with the approximate size of 1053 bp was amplified by utilizing the conditions (Fig. 1c).

Digestion of recombinant T-vector: Smar Taq polymerase adds adenine nucleotide at the 3' end of amplified fragment. Therefore, amplified fragment can insert into T-vector. Whereas, BamHI and NotI are endonuclease enzymes so will be more effective on inserted fragment into T-vector. As shown in Fig. 2 CCRL1 fragment was cut from T-vector with a suitable concentration.

In many studies was used T-vector. For example Shizuya and Goda were used T-vector in their researches (Goda *et al*., 2004; Shizuya *et al*., 1992). Hamiduddin determined the various expression level of this gene in different tissues using Northern blot. The maximum expression was demonstrated in heart tissue.

It is also express in other tissues as lungs, pancreas, spleen, ovaries, small intestine, fetal lung, liver and kidneys (Khoja *et al*., 2000). In this experiment, due to lack of access to human heart tissue, we have isolated CCRL1 gene from human spleen. We used *E. coli* (DH5α) cells for amplification of T-vector. Yung-Jiang have cloned *PACAP* gene, belonged to the GPCR into the pAlter1 vector and created site-directed mutagenesis in *PACAP* gene. As this study, they have amplified their recombinant vectors in *E. coli* bacteria (DH5α) (Yong-Jiang *et al*., 2000). Jo and Villalta were used NcoI, HindIII, EcoRI, BglII, PvuII, BstEII and PstI in their researches whereas we use BamHI and NotI in the research (Jo and Jo, 2001; Villalta *et al*., 1992).

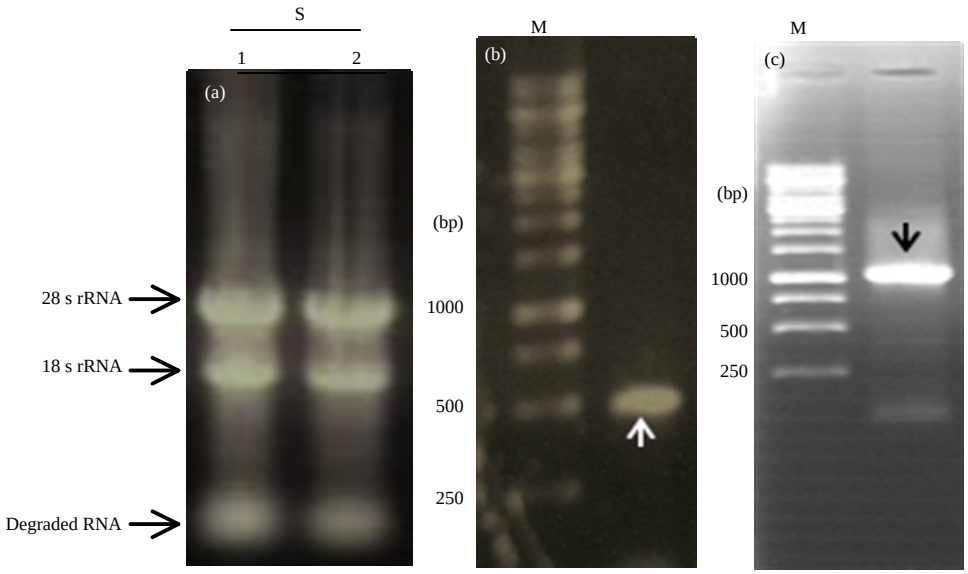

Fig. 1: Experimental steps for constructing CCRL1. a) RNA isolation from spleen tissue. b) RT-PCR product comprising the GAPDH cDNA which is shown by arrowhead. c) Agarose gel mobility of whole length of *CCRL1* gene, the amplified band is indicated by arrowhead. M: 1k bp DNA ladder

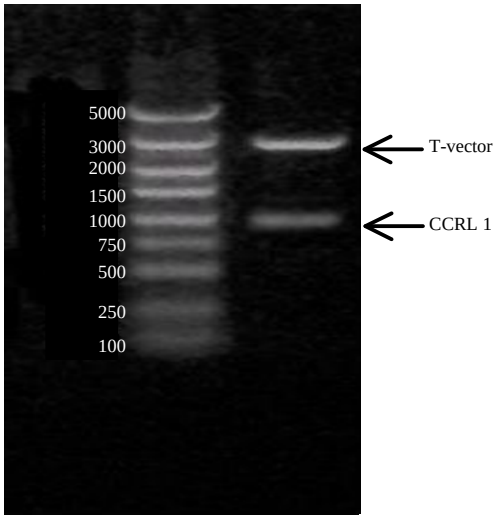

Fig. 2: Digestion of T-vector by BamHI and NotI. The CCRL1 fragment and T-vector is shown by arrowhead

CONCLUSION

In this study, bacteria that had received the recombinant vector formed white colonies on media containing X-gal and IPTG whereas other bacteria formed blue colonies. Recombinant vector was extracted and easily was digested by mentioned enzymes.

REFERENCES

Goda, N., T. Tenno, H. Takasu, H. Hiroaki, M. Shirakawa, 2004. The PRESAT-vector: Asymmetric T-vector for high-throughput screening of soluble protein domains for structural proteomics. Protein Sci., 13: 652-658.

Gu, Q., Y.S. Ding ans T.L. Zhang, 2010. Prediction of G-protein-coupled receptor classes in low homology using *Chou's pseudo* amino acid composition with approximate entropy and hydrophobicity patterns. Protein Pept. Lett., 17: 559-567.

Jo, C. and S. Jo, 2001. A simple method to construct T-vectors using *Xcm* I cassettes amplified by nonspecific PCR. Plasmid, 21: 235-240.

Joost, P. and A. Methner, 2002. Phylogenetic analysis of 277 human G-protein-coupled receptors as a tool for the prediction of orphan receptor ligands. Genome Biol., 3: 1-16.

Khoja, H., G. Wang, C.T. Ng, J. Tucker, T. Brown and V. Shyamala, 2000. Cloning of CCRL1, an orphan seven transmembrane receptor related to chemokine receptors, expressed abundantly in the heart. Gene, 246: 229-238.

King, N., C.T. Hittinger and S.B. Carroll, 2003. Evolution of key cell signaling and adhesion protein families predates animal origins. Science, 301: 361-363.

Qiu, J.D., J.H. Huang, R.P. Liang and X.Q. Lu, 2009. Prediction of G-protein-coupled receptor classes based on the concept of Chouspseudo amino acid composition: An approach from discrete wavelet transform. Anal. Bio., 390: 68-73.

Shizuya, H., B. Birren, U.J. Kim, V. Mancino, T. Slepak, Y. Tachiiri and M. Simon, 1992. Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in *Escherichia coli* using an F-factor-based vector. Proc. Natl. Acad. Sci. USA., 89: 8794-8797.

Villalta, M., P. Fernandez-Silva, B. Beltran, L. Enguita, M. Lopez-Perez and J. Montoya, 1992. Molecular characterization and cloning of sheep mitochondrial DNA. Biomed. Life Sci., 21: 235-240.

Xiao, X., W. Pu and C. Kuo-Chen, 2009. A cellular automaton image approach for predicting G-protein-coupled receptor functional classes. J. Comput. Chem., 30: 1414-1423.

Yong-Jiang, C., G. Gerald and F. Falk, 2000.. A mutation in the second intracellular loop of the pituitary adenylate cyclase activating polypeptide type I receptor confers constitutive receptor activation. FEBS. Lett., 469: 142-146.