Expression of MLAA34-HSP70 fusion gene constructed by SOE-PCR

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Abstract: To construct the pIRES2-MLAA34-HSP70 recombinant vector and express the MLAA34-HSP70 recombinant proteins in *Escherichia coli (E. coli).* The MLAA34 and the HSP70 genes were extracted from U937 cells by RT-PCR, and then we amplified the fusion gene MLAA34-HSP70 by SOE-PCR and inserted it into the pIRES2-EGFP vector to construct the pIRES2-MLAA34-HSP70 recombinant vector. We amplified the fusion gene MLAA34-HSP70 successfully and identified the correctness of pIRES2-MLAA34-HSP70 recombinant vector by PCR and restriction endonuclease. Moreover, the MLAA34-HSP70 recombinant proteins expressed in *E. coli* were consistent with the expected molecular weight. We constructed the pIRES2-MLAA34-HSP70 recombinant vector successfully and the MLAA34-HSP70 recombinant proteins were successfully expressed by the induction of IPTG.

Keywords: Micro residual disease, MLAA-34, HSP-70, SOE-PCR, DNA vaccine.

INTRODUCTION

The DNA vaccine is to prevent and cure disease by the transfer of a foreign gene into animal somatic cells, which is translated into antigenic proteins that induced the host immune response (Song *et al*., 2013). Epitope gene is the key part of DNA vaccine and the most commonly used epitope gene is the fusion gene, which can enhance the immunogenicity and improve the immune protective effect of DNA vaccine. Splicing by overlap extension PCR (SOE-PCR) is an effective way to build a fusion gene. Complementary primers were used to form overlapping bands of PCR products, and then multiple gene fragments from different sources are spliced together Horton *et al*., 1990. SOE-PCR technique is effective for gene recombination without restriction enzyme and is widely used to construct the fusion gene, fixed point mutation and gene knockout etc. (PENG *et al*., 2016). We want to construct DNA vaccine MLAA 34-HSP70 by SOE-PCR and study its immunogenicity in *E. coli*.

MATERIALS AND METHODS

Materials

Restriction enzymes *EcoR* I and *BamH* I, T4 DNA ligase, Total RNA isolatio kit, Plasmid extraction kit, RT-PCR kit and RPMI 1640 as supplemented with 10% FBS were all obtained from Sangon Biotech (Shanghai) Co., Ltd.

Primers design and synthesis

According to the length of MLAA-34 (Gen Bank: AY288977.2) and HSP-70 (GenBank: NM_005345.5) primers were designed. The full length of MLAA-34 gene was 1014 bp and HSP-70 was 1927bp. The overlapping sequences of primer P2 and P3 was GGCGGCGGCGG CGGC and the primers were synthesis by Sangon Biotech (Shanghai) Co., Ltd. (table 1.)

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Cell culture and gene extraction

U937 cells were cultured in RPMI 1640 as supplemented with 10% FBS and collected to isolate RNA for RT-PCR. PCR reaction conditions: 94°C 5 min, 94°C 30 s, 55°C 30 s, 72°C 60 s, 30cycles. PCR amplification products were recovered and purified.

SOE-PCR amplified MLAA34-HSP70 fusion gene

MLAA34-HSP70 fusion gene were amplified with template purified MLAA-34 and HSP70 genes by SOE-PCR. PCR reaction conditions: 94°C 5 min, 94°C 30 s, 55°C 30 s, 72°C 60 s, 30cycles. PCR amplification products were recovered and purified.

Construction of recombinant plasmid pIRES2-MLAA34-HSP70

MLAA34-HSP70 fusion gene and pIRES2-EGFP plasmid were digested by *EcoR* I and *BamH* I. And the recombinant plasmid pIRES2- MLAA34-HSP70 were constructed by T4 DNA ligase. The recombinant plasmid was transformed into *E. coli.* and identified by PCR and restriction enzymes *EcoR* I and *BamH* I.

Prokaryotic expression of recombinant plasmid pIRES2-MLAA 34-HSP70

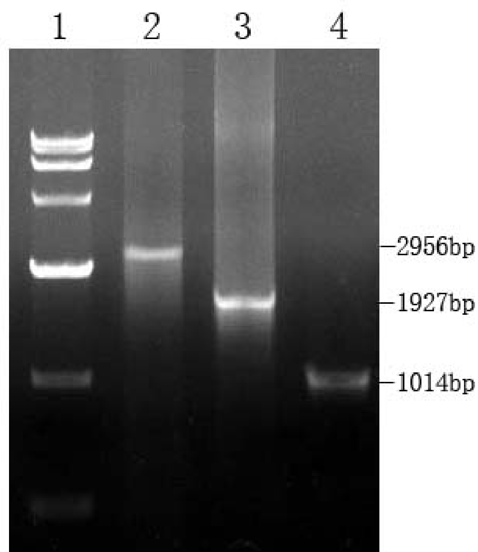
E. coli carrying recombinant plasmid pIRES2-MLAA34-HSP70 was inoculated in LB liquid medium. The MLAA34-HSP70 recombinant proteins were successfully expressed by the induction of IPTG (1.0mmol/L) for 3h and 5h respectively and were analyzed by western-blot.

RESULTS

MLAA 34-HSP70 fusion gene

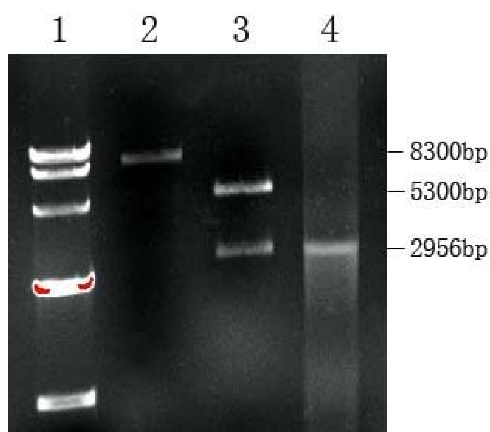
With the template MLAA-34 gene and HSP-70 gene, we

amplified MLAA34-HSP70 fusion gene by SOE-PCR as shown in fig. 1.



Note: 1, Marker; 2, MLAA34-HSP70 fusion gene 3, HSP70 gene; 4, MLAA34 gene

Fig. 1: MLAA 34-HSP70 fusion gene



Note: 1, Marker; 2, pIRES2-MLAA34-HSP70 plasmid; 3, Results of double enzyme digestion; 4, Identification results of PCR

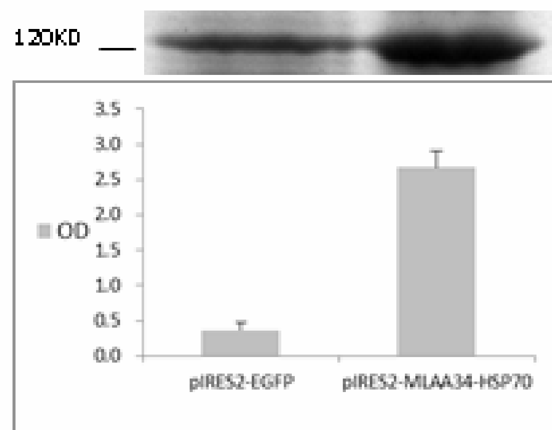
Fig. 2: Identification of pIRES2-MLAA34-HSP70

Construction and identification of pIRES2-MLAA 34-HSP70

The recombinant plasmid pIRES2- MLAA34-HSP70 were constructed by T4 DNA ligase. After transformation into *E. coli.*, we identified the recombinant plasmid by PCR and restriction enzymes *EcoR* I and *BamH* I as shown in fig. 2.

Western-blot of pIRES2-MLAA 34-HSP70 expressed in E. coli

The recombinant plasmid pIRES2- MLAA34-HSP70 was induced to express the fusion protein in *E. coli* and the expressed protein was analyzed by western-blot with anti-HSP70 as the primary antibody (fig. 3.).



Note: 1, pIRES2-EGFP plasmid. 2, pIRES2-MLAA34-HSP70 plasmid

Fig. 3: Identification MLAA34-HSP70 recombinant proteins by Western-blot.

DISCUSSION

DNA vaccine is a simple and safe treatment of acute leukemia. DNA vaccine can be uptaken by antigen-presenting cells when it is injected into the host body and antigen protein will be expressed in cells. The antigen protein can stimulate the body to produce cellular immunity and humoral immunity (Donnelly *et al*., 2005). Epitope gene is the key part of DNA vaccine to antitumor. MLAA-34 gene is a good target gene for acute leukemia immunotherapy because it is closely related to the occurrence of acute mononuclear cell leukemia and its down-regulation can induce leukemia cell apoptosis (Zhao *et al*., 2011; Zhang *et al*., 2013; ZHAO *et al*., 2016). HSP70 is a factor of tumor gene regulation which can induce specific anti-tumor immune response in bodies. So we amplified MLAA34-HSP70 fusion gene as the Epitope gene by SOE-PCR to construct the DNA vaccine (You 2012, Guzhova and Margulis 2016).

Overlapping sequences of primer are the most important part of SOE-PCR and the overlapping sequences would be a bridge to ligate two genes by PCR. The recombination efficiency of SOE-PCR is higher than that of restriction enzyme (Dahm and Jennewein 2010). SOE-PCR is widely used in vaccines, polyclonal antibodies, and genetic engineering in plants, etc. (Zhang *et al*., 2015; Yuelan *et al*., 2016; Li *et al*., 2014). We used flexible peptide GGCGGCGGCGGCGGC as the overlapping sequences to design primer, which could keep the function of each part of the fusion protein, such as MLAA34 and HSP70. It is showed in our experiments that DNA vaccine pIRES2-MLAA 34-HSP70 was constructed successfully and expressed MLAA34-HSP70 fusion protein in *E. coli.* which is detected by western-blot. The MLAA34-HSP70 fusion protein has good antigenicity and can be used for further studies.

Table 1: Primers sequences

Primers	Sequences
P1	GCGAATTCATGAAAAAAATGCCTTTGTTTAGT
P2	GGCGGCGGCGGCGGCTCAAGGGGCCGTTTTCTTCAAG
P3	GGCGGCGGCGGCGGCATGGCCAAAGCCGCGGCGATC
P4	CGGGATCCCTAATCTACCTCCTCAATGGTG

In short, SOE-PCR is a good method to construct fusion gene in DNA vaccine, which is widely used in immunotherapy. Our study laid a foundation for further tumor immunotherapy studies.

REFERENCES

Dahm P and Jennewein S 2010). Introduction of the early pathway to taxol biosynthesis in yeast by means of biosynthetic gene cluster construction using SOE-PCR and homologous recombination. *Methods Mol. Biol.,* **643**: 145-163.

Donnelly JJ, Wahren B and Liu MA (2005). DNA vaccines: Progress and challenges. *J. Immunol.,* **175**(2): 633-639.

Guzhova IV and Margulis B (2016). HSP70-based anti-cancer immunotherapy. *Hum Vaccin. Immunother.,* **13**: 1-7.

Horton RM, Cai ZL and Ho SN *et al* (1990). Gene splicing by overlap extension: Tailor-made genes using the polymerase chain reaction. *Biotechniques,* **8**(5): 528-535.

Li B, Ye J and Lin Y *et al* (2014). Preparation and identification of a single-chain variable fragment antibody against Newcastle diseases virus F48E9. *Vet Immunol. Immunopathol.,* **161**(3/4): 258-264.

PENG Lei, ZHAO Yan and MAYin-hua (2016). Optimization of Splicing by Overlap Extension PCR Using Nested PCR. *J. of Anhui Agri. Sci.,* **44**(20): 126-127.

Song Xiaohui, Chen Hongsuo and Sun Yanan *et al* (2013). Research progress on the leukemia vaccine. *Journal of Jilin Medical College,* **34**(3): 208-212．

You Chaoyong (2012). Research on Relationship between HSP70 and Malignant Tumor. *The Practical Journal of Cancer,* **27**(1): 89-91.

Yuelan Z, Yiwei L and Liyuan L *et al.* (2016). Expression and identification of the ADF-linker-3-1E gene of *Eimeria acervulina* of chicken. *Parasitol. Res.,* **115**(4): 1641-1647.

Zhang WJ, Zhang WG and Zhang PY *et al* (2013). The expression and functional characterization associated with cell apoptosis and proteomic analysis of the novel gene MLAA-34 in U937 cells. *Oncol. Rep.,* **29**(2): 491-506.

Zhang M, Jiang S and Zheng J *et al* (2015). Construction of an integration vector carrying hygromycin B resistance gene and its genetic transformation in Rhizopus oryzae. *Sheng wu gong cheng xue bao Chinese Journal of Biotechnology,* **31**: 1203-1218.

Zhao J, He A and Zhang W *et al* (2011). Quantitative assessment of MLAA-34 expression in diagnosis and prognosis of acute monocytic leukemia. *Cancer Immunol. Immunother.,* **60**(4): 587-597.

Zhao Tianyu, Zhao Jiaqi and Fan Yening *et al* (2016). Construction of pIＲES2-MLAA34-EGFP vector and its expression. *Journal of Jilin Medical College,* **3**:184-187.