

# Gene cloning of P43 surface protein of toxoplasma gondii tachyzoite and bradyzoite (SAG3)

## Research Article

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**Abbreviations:** glycosyl phosphatidyl inositol, (GPI); low melting point, (LMP); P43 surface antigen, (SAG3)

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## Summary

**Toxoplasma gondii is an obligate intracellular parasite which its sexual and asexual cycle respectively takes place in the intestinal epithelial of definitive host and tissue of intermediate hosts. Congenital toxoplasmosis is more important when the mother acquired the infection during pregnancy period for the first time. Having a specific antigen is an important element in prevention and detection of parasite. This study has designed and performed in the aim of cloning a specific toxoplasma antigen for further studies. We have amplified gene of P43 of toxoplasma tachyzoite and bradyzoite surface antigen. PCR product was cloned in pGEMEX1 expression vector (named pGEM43) and is ready to make the recombinant protein for using as antigen.**

## I. Introduction

Toxoplasma gondii is an obligate intracellular parasite and its life cycle includes definitive and intermediate hosts. The sexual and asexual cycle of parasite respectively takes place in the intestinal epithelial of the cat (as definitive host) and any warm blooded, like mammals and birds (as intermediate hosts) (Frankel et al, 1970). Previous studies have showed that the main human infection can result by ingestion of material contaminated with infected cat feces, from eating raw or partially cooked beef and transplacental transmission from mother to children (Miller et al, 1972). Congenital toxoplasmosis is more important in the pregnant women who acquired the infection for the first one (Guerina 1994). Human infection takes place in two forms: acute infection and chronic infection. After beginning of the infection with initial immune response, tachyzoite (multiply fast) escape to different tissue via blood and lymph, then invert to bradyzoite (multiply slowly) inside tissue cyst (Hutchison 1970).

Recently, main attention has been attracting to surface molecules of parasite. The surface of tachyzoite

and bradyzoite have covered with antigens which is linked to GPI (glycosyl phosphatidyl inositol) (Nagel and Boothroyd 1989; Tomavo et al, 1989) that are known as SAG (surface antigens) (Boothroyd et al, 1998; Lekutis et al, 2000). Some of these specific molecules are specified stage of parasite life cycle: 30KD (SAG1), 22KDa (SAG2) and 35KDa (SRS3) proteins are only in the surface of tachyzoite and are not in the surface of bradyzoite, meanwhile some other proteins such as 43KD (SAG3) and 23KDa are in the surface of both tachyzoite and bradyzoite (Burg et al, 1988; Prince et al, 1990; Manger et al, 1998; Lekutis et al, 2001). More studies are done about the cloning and expression of genes of toxoplasma surface proteins (Burg et al, 1988; Prince et al, 1990; Cesbron-Delauw et al, 1994). The toxoplasma P43 have been cloned and sequenced first by Cesbron-Delauw and colleagues in 1994 and additionally by Fux and colleagues in 2003. The aim of this study was cloning the gene of P43 surface antigen (SAG3) of toxoplasma tachyzoite and bradyzoite as a recombinant protein.

## II. Materials and Methods

### A. Parasite

Toxoplasma RH strain, kindly provided by Professor Dalimi Tarbiat Modarres University, Tehran, Iran and was maintained by twice-weekly passages of peritoneal fluid into mice. Toxoplasma tachyzoites were isolated from peritoneum puncture of infected mice and were rinsed by PBS buffer many times. Toxoplasma DNA was extracted as previously described (Zia-Ali, 2005).

### B. PCR reaction

We designed a set of primer for amplification of P43 gene with SacI and BamHI restriction sites at 5' end of forward and reverse primers respectively (Tox43 F 5'-GAGCTCATATGCAGCTGT GCGGC GC-3' and Tox43 R 5'-GGA TCCTTA GGCAGC CACATGCAC-3). PCR reaction contained 0.5 µg DNA, 40 pico mol each of forward and reverse primers, 1.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 1X PCR buffer, 1.5 unit of Taq DNA polymerase (CinnaGen, Iran) and dH<sub>2</sub>O up to 50 µL. PCR amplification was carried out with 30 cycles of denaturation at 94 °C for 40 seconds, annealing at 65 °C for 60 seconds and extension at 72 °C for 60 seconds. PCR reaction was incubated at 94 °C and 72 °C for 5 min before and after the PCR cycling respectively (Pherson et al 2000).

### C. Electrophoresis

PCR product was submitted to electrophoresis using 1% agarose gel and stained by ethidium bromide. The DNA band was visualized under ultraviolet light (UV transilluminator) (Boffey 1984).

### D. Gene cloning

PCR product was electrophoresed on 1% low melting point (LMP) agarose gel (Gaastra and Jorgensen, 1984) and DNA band was sliced under long wave UV and recovered by DNA purification kit (Fermentas, Cat. No k0513). Recovered DNA was cloned in pBluescript cloning vector via T/A cloning method. Briefly, EcoRV blunt digested pBluescript was 3' tailed

using dTTP by terminal deoxy nucleotidyl transferase (Gaastra and Klemm, 1984; Eun, 1996) and 3' A tailed PCR product was ligated to it (Gaastra and Hansen, 1984). The ligation reaction was transformed in Ecoli XLI-blue strain competent cells (Hanaham, 1983) and dispensed on LB agar plate containing 50 µg/ml ampicillin. Colonies were screened by X-gal and IPTG and white colonies containing recombinant plasmid were selected (Bothwell et al 1990).

Recombinant plasmid was digested by SacI and BamHI and released expected DNA band was recovered by DNA purification kit (Fermentas Cat. No k0513) and subcloned in SacI and BamHI digested pGEMEX 1 expression vector. Reaction was transformed and colonies contained recombinant plasmids were mass cultured on LB medium. Recombinant plasmids were extracted and confirmed by restriction analysis.

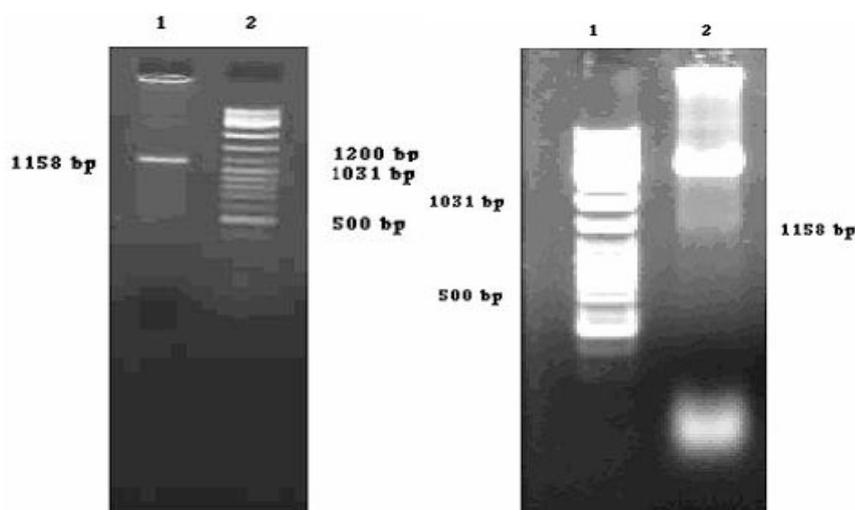
## III. Results

Toxoplasma tachyzoites were isolated by peritoneum puncture of infected mice and rinsed by PBS buffer. DNA was extracted and PCR reaction was carried out. **Figure 1** shows 1158 bp as PCR product of toxoplasma P43 gene.

PCR product was ligated in pBluescript via T/A cloning method and recombinant plasmid digested with SacI and BamHI restriction enzyme, **Figure 2** shows digested recombinant plasmid.

Digestion reaction was electrophoresed on LMP agarose gel, released DNA band was purified by DNA purification kit and subcloned in SacI and BamHI digested pGEMEX1 expression vector and named pGEM43. PstI enzyme has a restriction site at position 748 on P43 sequence but pGEMEX1 don't cut by this enzyme. For confirmation of recombinant pGEM43 we digested recombinant plasmid by PstI and lineared plasmid is shown in **Figure 3**.

Gene was sequenced and deposited in GeneBank at accession no. EF445545



**Figure 1.** 1% agarose gel electrophoresis. Lane 1: 1158 bp as PCR product of P43. Lane 2: 100 bp DNA ladder marker.

**Figure 2.** 1% agarose gel electrophoresis. Lane 1: 100 bp DNA ladder marker. Lane 2: Digested recombinant plasmid by SacI and BamHI restriction enzymes.

**Figure 3.** Confirmation of recombinant plasmid. Lane 1: Recombinant plasmid (digested by PstI). Lane 2: No recombinant plasmid (not digested by PstI).

## IV. Discussion

*Toxoplasma gondii* is an obligatory intracellular parasite which has complicated life cycle. Sexual and asexual cycle respectively takes place in intestinal epithelial cells of cat and tissues of mammals and birds (Frankel et al, 1970). This parasite almost attacks to all host nucleated cells (Sibley, 1995). *Toxoplasma gondii* leads to dangerous manifestation in fetus. The most dangerous effect of congenital toxoplasmosis some times is abortion and premature delivery (Dunn, 1999; Freeman, 2005).

The congenital infection according to the intensity and variety of the organs contamination has different symptoms. Difference in the intensity of the disease depends on the stage of the pregnancy period which the infection occurs (Zhao 1992; Wallon et al, 2002). This parasite will be detected in human beings by serological tests only, and specific antigen is very essential in diagnosis system. P43 (SAG3) is one member of the redundant system of *T. gondii* receptors that act as ligands mediating host cell recognition and involved in the parasite attachment to target cells (Jacque 2001, Dzierszinski, 2000). In this study for availability of parasite stage specific antigen, the gene of p43, the tachyzoite and bradyzoite surface antigen was cloned and became ready to make recombinant proteins. It can be used as antigen for detection or prevention of parasite in men.

## V. Conclusion

In this study, the gene of P43 toxoplasma tachyzoite and bradyzoite surface antigen was cloned in expression vector and confirmed. It can be used for either diagnosis or prevention of parasite in men.

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