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# Construction of recombinant plasmid containing apoptin gene

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## Abstract

Apoptin, a protein known to selectively induce apoptosis in cancer cells, was successfully released from a recombinant pTargeT vector and cloned into the pVAXI vector. This approach was thoroughly confirmed using a variety of molecular biology techniques. Restriction enzyme analysis confirmed that the Apoptin gene was present and correctly oriented within the pVAXI vector. Polymerase Chain Reaction (PCR) amplification confirmed that the gene had been successfully inserted. Furthermore, sequencing of the cloned construct confirmed that the Apoptin gene was properly orientated and undamaged. These rigorous verification methods confirm the cloning process's dependability and precision, paving the door for future research into Apoptin's medicinal potential in cancer treatment.

## Introduction

Chicken anemia virus (CAV) is a significant pathogen in poultry, causing a wide range of clinical manifestations that can lead to substantial economic losses in the poultry industry. The genome of CAV is a negative-strand DNA of approximately 2.3 kilobases (kb) (Todd et al., 2007). This small yet potent genome encodes several proteins, among which Apoptin has garnered considerable attention due to its unique properties.

Apoptin, a 13.6 kDa protein, has been shown to induce apoptosis specifically in transformed and tumor cells, while leaving normal cells unaffected (Danen-Van Oorschot et al., 1997). This selective cytotoxicity makes Apoptin a promising candidate for cancer therapy. The mechanism by which Apoptin induces apoptosis is not fully understood, but it is believed to involve multiple pathways, including the activation of caspases and the disruption of cellular signaling networks (Noteborn, 2004).

The initial discovery of Apoptin's apoptotic effects was made in chicken thymocytes and chicken lymphoblastoid cell lines (Jeurissen et al., 1992). These findings laid the groundwork for further investigations into the potential of Apoptin as a therapeutic agent. Subsequent studies have demonstrated that Apoptin can induce apoptosis in a variety of human cancer cell lines, including those derived from breast, lung, and colon cancers (Pietersen et al., 1999).

The selective nature of Apoptin's cytotoxicity is particularly intriguing. It has been hypothesized that this selectivity is due to the differential localization of Apoptin in transformed versus normal cells. In transformed cells, Apoptin is predominantly found in the nucleus, where it can interact with various nuclear proteins and induce apoptosis. In contrast, in normal cells, Apoptin remains largely in the cytoplasm, where it is unable to exert its apoptotic effects (Danen-Van Oorschot et al., 2000).

Understanding the molecular mechanisms underlying Apoptin's selective cytotoxicity is crucial for its potential application in cancer therapy. Recent advances in molecular biology and genomics have provided new tools and techniques for studying the interactions between Apoptin and its cellular targets. These studies have the potential to uncover novel therapeutic strategies for the treatment of cancer and other diseases characterized by aberrant cell proliferation.

In summary, the chicken anemia virus genome, despite its small size, encodes proteins with significant biological and therapeutic potential. Apoptin, in particular, has emerged as a promising candidate for cancer therapy due to its ability to selectively induce apoptosis in transformed cells. Further research into the molecular mechanisms of Apoptin's action is essential for harnessing its full therapeutic potential and developing effective strategies for cancer treatment.

## Materials and Methods

### Plasmid

pVAXI and apoptin containing plasmid were obtained from BTS Institute of Science & Technology, Mundia Ahmednagar, Pilibhit Road, Bareilly-243122, UP.

The forward primer F 5' ATG AAC GCT CTC CAA GAA G3' and reverse primer R 5' CTT ACA GTC TTA TAC ACC TT 3' were employed.

### Construction ofrecombinant plasmid

pVAX1 and pTargeT containing apoptin were cut with NheI and KpnI at 37C overnight, and then run on 1.5% agarose gel, examined under UV transilluminator and both pVAX1 and apoptin DNA band were cut and kept in separate microfuge tubes. These were extracted from gel using phenol-chloroform. pVAX1 was dephosphorylated with calf intestinal alkaline phosphatase. Ligation mixture was prepared with 1µl pVAXIDNA, 2µl apoptin DNA, 1µl T4 DNA ligase, 1µl ligase buffer and 5µl nuclease free water in a microfuge. It was mixed, microcentrifuged and kept overnight at 14C.

### Transformation

The *E. coli* DH5 was processed and transformed with ligation mixture as described by (Chung et al, 1989), transformants were spread on LB agar plate with 25µg/ml kanamycin andkept at 37C overnight. Twelve12 colonies were selected and plasmid DNA isolated by method of He et al (1990).plasmid DNA was isolated using TELT method (He et al, 1990).

### DNAstar analysis

DNAstar analysis of recombinant pVAX1 DNA sequence revealed that PstI enzyme was suitable to confirm right orientation of the apoptin which is expected to produce fragments of size 3000bp and 345 bp.

## PCR

Apoptingene in right orientation wasfurther confirmed by PCR using T7 forward primer and apoptin reverse primer. Sequencing with T7 primer also done to confirmapoptin right orientation.

## Results and Discussion

The apoptin gene was removed recombinant pTargeT (Fig. 1) It was ligated to pVAXIand the ligation mixture transformed E. coli DH5 alpha were plated on LB agar containing kanamycin. Twelve colonies wereselected, plasmid DNA isolated, digested with PstIand the one producing two fragments of size 3.026 kb and 0.345 kb (Fig. 2) was selected.These sizes were expected if the insert is in right orientation and thus it was confirmed that the VP3 gene was in right orientation. Colony PCR with T7 forward primer and apoptin reverse primer yielded 372bp product which confirmed the correct orientation (Fig. 3). Sequencing with T7 primer further confirmed the gene in right orientation.

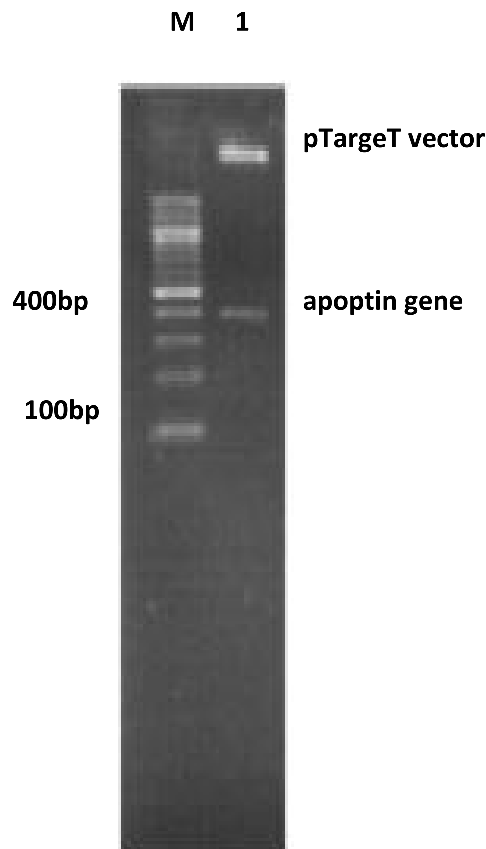


**Figure 1. pTargeT.ca-vp3 digested with Nhel and KpnI. Lane M :100 bp DNA ladder, 1: apoptin gene and pTargeT .**

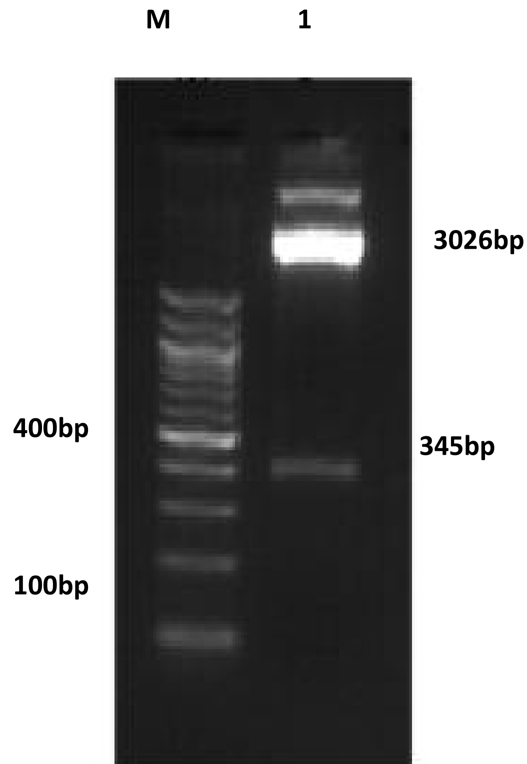


**Figure 2. pVAXI.cav-vp3 cut with PstI releasing fragments confirming right orientation,Lane M :100 bp ladder, 1: 3.026 bp and 345 bp fragments**

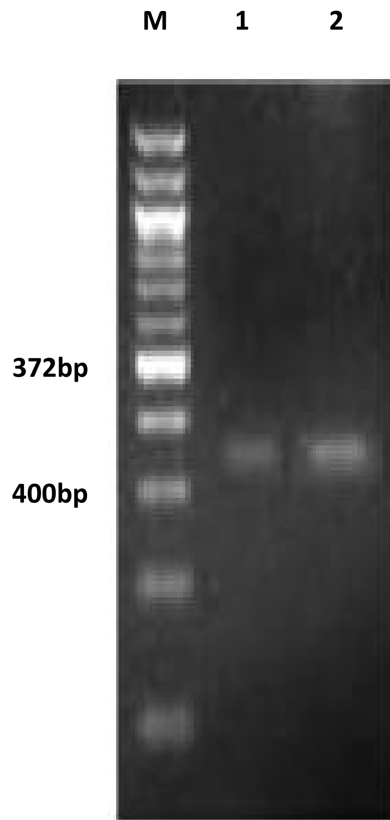


**Figure 3. PCR amplification of apoptin gene from transformed colony. Lane M: 100bp DNA marker, 1&2: 372 bp apoptin gene.**

Lee et al, (2007) demonstrated the effect of apoptin on canine mammary tumor cells. Natesan et al, (2006) showed anti-neoplastic activity of apoptin. Anti-neoplastic property of apoptin was also shown by other workers (Lee et al, 2012; Wu et al, 2012).

## Conclusion

Apoptin from chicken anemia virus was successfully recloned in pVAX1 vector.

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