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# Cloning of canine adenovirus 2 hexon gene in pAlpha vector

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## ABSTRACT
**Canine adenovirus-2 hexon gene was removed from recombinant plasmid pTarget-cav2-hex and recloned in replicon based pAlpha vector at StuI site in right orientation. It can be used in developing DNA vaccine in further studies.**

**Key words: Cloning, adenovirus, hexon, gene, plasmid**

## INTRODUCTION
Canine adenovirus (CAV), a non-enveloped dsDNA virus, is a member of the genus Mastadenovirus under the family Adenoviridae. Based on genetic and antigenic characteristics, canine adenoviruses are divided into two types: canine adenovirus type 1 (CAV-1) and canine adenovirus type 2 (CAV-2). Although CAV-1 affects the digestive tract and causes infectious canine hepatitis (ICH) accompanied by uveitis and interstitial nephritis in dogs, CAV-2 is mainly associated with respiratory type of disease. Saxena et al.,(2008) reported that a Sindbis virus replicon-based DNA vaccine encoding rabies virus glycoprotein (G) was developed by subcloning rabies G gene into a Sindbis virus replicon-based vaccine vector (pAlpha). Cloning of CAV-2 hexon gene in pAlpha vector has future perspective and potential to be used for the development of novel viral vaccines and therapeutics and it provides a wide range of advantages for researchers. Therefore it is a valuable tool for genetic engineering and research.

## MATERIALS AND METHODS
### Plasmid Vector
pTargeT-cav2-hex recombinant plasmid and the pAlpha vector containing replicase gene of Sindbis virus were available in Biotechnology Lab of this Institute (Salunkhe et al, 2008; Saxena et al, 2008; Sandey et al, 2008). The E. coli DH5α containing recombinant plasmid pTarget-cav2-hex (canine adenovirus-2 hexon) gene was freshly inoculated in LB broth containing ampicillin and incubated overnight at 37C. The isolation of plasmid DNA was done by TELT method (He et al., 1990).

### Cloning of cav-2-hex gene in pAlpha vector
The restriction enzyme digestion of pTargeT-cav2-hex plasmid DNA was done with EcoRI to release the gene insert which had no EcoRI site in it. A 50µl reaction mix was prepared containing pTargeT- cav-2 hex DNA 30 µl, EcoRI Buffer (10x) 5 µl, EcoRI Enzyme 2 µl, distilled water 13 µl, mixed by tapping the tube, centrifuged the tube at 5000 rpm for 15 sec and incubated at 37°C overnight in incubator. Agarose gel electrophoresis using 0.7% LMT agarose using 1x TAE buffer was done and the insert was cut out and collected in a microfuge tube. Blunt ending of DNA overhang (end filling) was done by adding dH2O 5 µl, 10 mM dNTPs mix 5 µl, T4 DNA Polymerase buffer 2 µl, T4 DNA Polymerase (6 µg) 1 µl, mixing and incubating at 37˚C for 30 min.

### Preparation of pAlpha vector

A blunt end cut was made using a 50 µl reaction mix of distilled water 18 µl, pAlpha vector DNA 25 µl, StuI buffer 5 µl, StuI enzyme 2µl, mixed by tapping, spin briefly and incubated at 30˚C for 2 h. For dephosphorylation, extracted DNA by phenol:chloroform, precipitated with ethanol and resuspended DNA in TE pH 8.0, added 10 µl 10X CIP buffer and the appropriate amount of calf intestinal phosphatase (CIP), incubated 37ºC 15 min then 55ºC 45 min. Inactivated the phosphatase activity at the end of the incubation period by heating to 65ºC for 30 min and the it was extracted with phenol/chloroform and recovered the DNA by standard precipitation with ethanol (Rai et al.,2020).

**Ligation**

For ligation, the 10 µl reaction mix contsined DW 4 µl, pAlpha vector (StuI cut) 1 µl, cav-2 hex insert DNA 2 µl, 10X Ligase buffer 1 µl, T4 DNA Ligase (2U) 1µl,10 mM ATP 1 µl. It was incubated at 14C for overnight. Centrifuged the ligation reaction briefly and place on ice.

**Transformation**

Used one step method of competent cell preparation and transformation (Chung et al, 1989) to transform *E. coli* DH5α.Transformants were selected by plating cells (in triplicate) on LB agar plates containing ampicillin 50µg/ml final conc and plasmid DNA isolation was done from 16 randomly selected colonies.

**Selection of clone containing gene in right orientation**

To check the right orientation of CAV-2 hexon gene in pAlpha vector we used DNAstar software MapDraw program. The pAlpha vector size is 10.779 kb, CAV-2 hex gene size is 2.736 kb totalling to 13.515 kb. The rplasmid cut with sph1 if in right orientation will yield fragments of 12.907 kb, 0.566 kb and 42 bp (not seen) while in wrong orientation it will yield fragments of 10.821 kb, 2.128 kb and 566 bp.

## RESULTS AND DISCUSSION

**Cloning of CAV-2- hexon gene into pAlpha vector**

The E. coli DH5α containing pTargeT-cav-2 hex recombinant plasmid as well as the one containing pAlpha plasmid vector were revived successfully. Plasmid DNA were isolated from both cultures.The rplasmid pTargeT-cav2-hex was digested with EcoRI to release the insert and it was successfully cut out from the gel (Fig 1). It was blunt ended, dephosphorylated and purified. Similarly the plasmid DNA pAlpha was isolated, digested with StuI to produce blunt ends and was prepared by dephosphorylation and purification. Ligation mixture was prepared and processed and competent E.coli DH5α cells were transformed and plated on agarose LB broth containing ampicillin 100µg/ml final concentration and incubated overnight at 37C. Large number of colonies were observed. Sixteen colonies which were individually isolated were randomly picked up and transferred to 3ml LB broth containing ampicillin in each tube and incubated at 37C overnight. The plasmid DNA was isolated from each colony, digested with SphI restriction enzyme, mixed with loading dye and run on 0.7% agarose gel submarine horizontal electrophoresis using TAE buffer. The gel was examined in transilluminator and photographed (Fig 2). It was observed that clone 7 contained the gene in right orientation since a band at 12.9kb along with 0.566kb band could be seen which was expected as analysed using DNAstar MapDraw software.

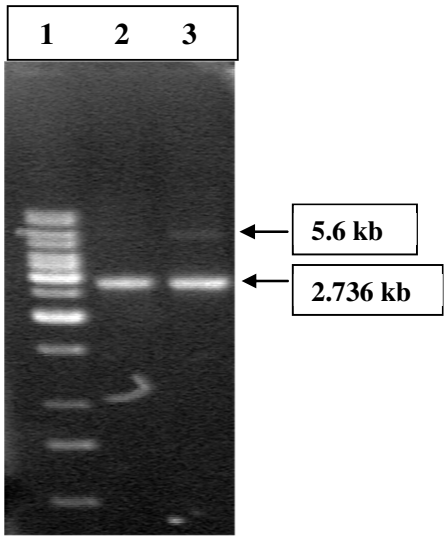


**Fig.1. pTargeT-cav2-hex digested with EcoRI. Lane 1, 1kb DNA ladder, Lane 2,3: 2.736 kb cav2-hex gene insert and 5.67 kb pTargeT vector.**

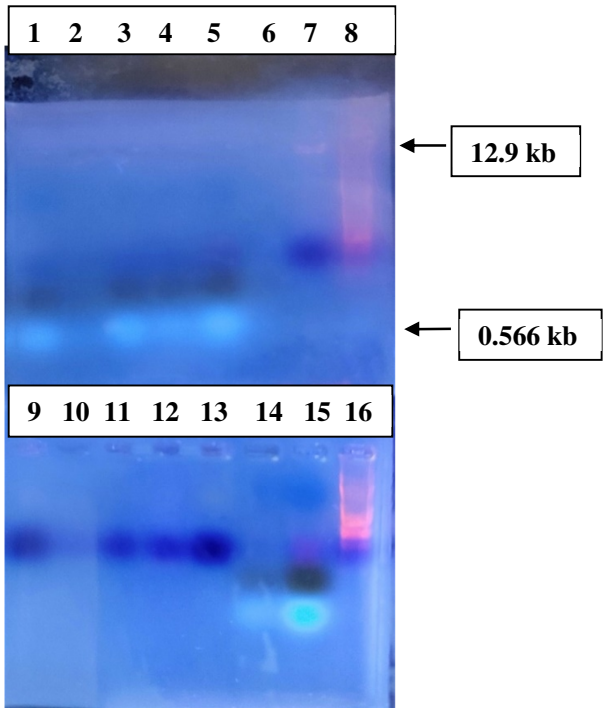


**Fig.2. Plasmid DNA from transformed colonies cut with SphI. Lane 1-7, pDNA, clone 7 shows DNA band at 12.9kb indicating that gene insert is in right orientation. Lane 8,16: DNA MW marker EcoRI HindIII double digest. Lane 9-15 transformed colonies pDNA. Lane 5, 12, 15 shows gene in wrong orientation.**

**Analysis of orientation of CAV-2 hexon gene in pAlpha vector**

The present experiments have yielded a recombinant plasmid pAlpha-cav-2-hex which is very valuable for high level of gene expression and use in DNA vaccine trials. The pAlpha vector contains replicas gene of Sindbis virus which maked 10,000 t0 100,000 copies of the insert gene and thus it will be agreat asset in using it as DNA vaccine candidate. Of course for this, field trial using appropriate protocol is needed which can be taken up in a separate investigation. Since it will thus require very small amount of plasmid DNA, 1µg or even few nanogram to induce immunity, it will be very useful in a country like India where population is very large and economic considerations play a crucial role. In addition to its safe and efficacious use, as detailed above, it provides important features including, humoral and cellular immune responses to vaccine, which viral pathogen targets might require for protection.

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