

Short Report

## Designing E1 Deleted Adenoviral Vector by Homologous Recombination

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### ABSTRACT

**Background:** Adenoviruses are used extensively to deliver genes into mammalian cells, particularly where there is a requirement for high-level expression of transgene products in cultured cells, or for use as recombinant viral vaccines or in gene therapy. In spite of their usefulness, the construction of adenoviral vectors (AdV) is a cumbersome and lengthy process that is not readily amenable to the generation of large collection of clones. **Methods:** In this project, to delete E1 gene in adenovirus, an adenoviral plasmid containing lateral sites of E1 region of adenovirus was made and recombination in the 293A cells between the homologous region of this linearized plasmid and the adenovirus genome resulted in the formation of the complete adenoviral recombinant. **Results:** This recombination resulted in loss of E1 region and we constructed a recombinant adenovirus type 5 vector that E1 gene was deleted by homologous recombination. **Conclusion:** Homologous recombination is more easy and fast technique in the production of AdV. *Iran. Biomed. J. 11 (3): 199-202, 2007*

**Keywords:** Adenoviral vectors (AdV), Adenovirus, Homologous recombination

### INTRODUCTION

Adenoviruses are common DNA viruses that cause upper respiratory disease in human. The double-strand DNA genome of adenovirus is 36 kb, which can be manipulated by standard recombinant technology. The replication cycle of adenovirus has two distinct phases: early and late. The early transcription units, designated E1A and B, E2A and B, E3 and E4, are mainly active before the onset of DNA replication. Genes from E1, E2 and E3 encode essential regularly proteins involved in the transactivation of viral and cellular promoters, DNA replication, cell cycle and apoptosis [1-3].

Since their development in the early 1980s, adenoviral vectors (AdV) have been widely used in gene transfer experiments for vaccination, gene therapy and functional studies. Among the main

features that make AdV ideal gene transfer vectors are their efficient transgene expression in a wide range of cell types from various species both *in vitro* and *in vivo*, well characterized genome, no associated with human malignancy, stability of the recombinant variants and easy substitution of large portion of viral DNA by foreign sequences [4-8].

In spite of their usefulness, the construction of AdV is a cumbersome and lengthy process that is not readily amenable to the generation of large collection of clones. AdV development in the more classic gene delivery approach has focused on crippling the virus by deleting essential genetic regions and complementing the missing function(s) *in trans* using engineered cell lines or viral helper system. In this study, we constructed a rendered replication deficient adenovirus by deleting E1 region essential genes by homologous recombination.

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## MATERIALS AND METHODS

**Construction of adenoviral plasmid.** The pAdCMV5-BFPq (Kindly provided by Prof. Massie, Institute of Biotechnology Research, National Research of Canada) was digested by BglII and electrophoresed on the low-melting agarose gel. The band (5385 bp) was excised, purified and ligated to bring the adenovirus E1 lateral homology sites together [6, 9, 10].

**Construction of E1 deleted AdV by Co-transfection.** Anchorage dependent 293A ( $3 \times 10^5$ ) cells were plated in a 60-mm dish one day prior to transfection using DMEM medium. Same amount (2.5  $\mu$ g) of sterile linearized adenoviral plasmid with adenoviral DNA (AdE1+, PS+, E3+,  $\Delta$ E4 (orf6+) Rlu-GFP) was mixed and transfected onto sub-confluent 293A cells using polyethylenimine in hepes buffer (pH 7.2). After overnight incubation, the cells were split 1/3 and left to attach. Then, the medium was carefully and completely removed and the cell layered with 3 ml DMEM containing agarose 1.25%. To maintain the cell viability, 1-2 ml of DMEM containing agarose was added to the dish every 4-5 days [6, 11].

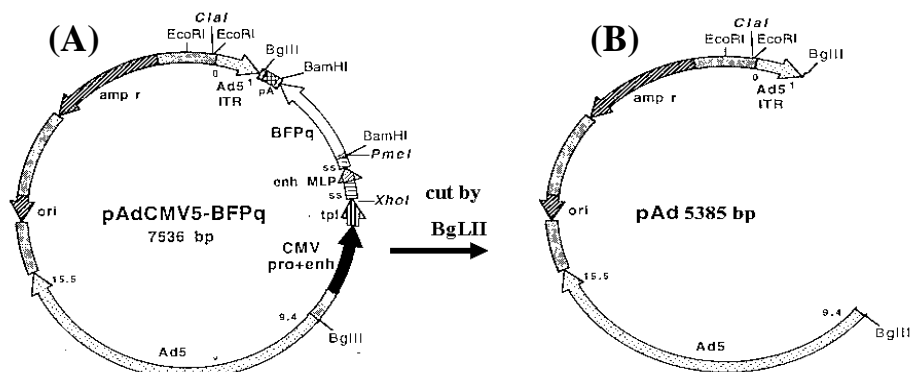
**Plaque elution, purification and amplification.** Well-isolated plaques were picked up (as agarose plugs) and eluted overnight at 37°C in 0.5 ml per well of DMEM in a 24 well plate. A volume of 100  $\mu$ l of elutes (about  $10^3$  virus particles) was used to infect 293A cells ( $5 \times 10^4$  per well) in 24 well containing 1 ml of DMEM until complete green fluorescence was obtained. The whole plate was frozen (-20)/thawed (37°C) 3 times and the resulting

solution was called the viral stock of the first amplification. Three strongly positive fluorescent plaques selected for plaque purification [12]. The 293A cells ( $3 \times 10^5$ ) were plated in each wells of a  $3 \times 6$ -well plate one day prior to infection using DMEM. It was assumed that a plaque contains between  $10^3$  and  $10^4$  pfu/ml so the virus was diluted in DMEM in six, 10 fold dilutions ( $10^{-2}$ - $10^{-7}$ ) to obtain 1-10 plaques per plate. The medium from each well of 293A cells was aspirated and added 1 ml of diluted virus to each well. The plates were incubated 6 to 7 hours. Then, the medium was aspirated from the wells, and then overlaid each well with 3 ml medium containing of 1.25% agarose. After about 10 days, well isolated plaques were picked and again were eluted and purified 3 times and were amplified.

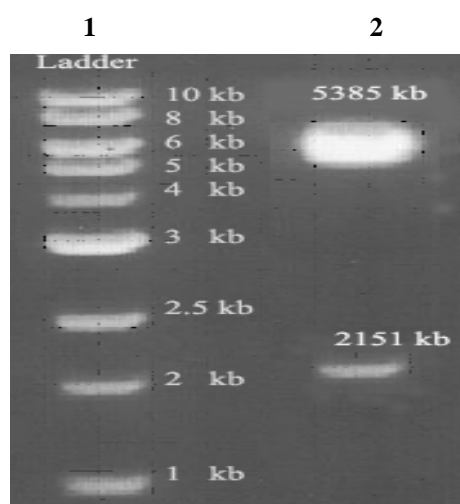
**DNA analysis in the E1 deleted AdV.** Viral DNA of AdV derived from the plaques were extracted and analyzed by NotI and XbaI restriction enzymes using the conditions recommended by the enzymes vendor (Fermentas, USA). The digested DNA was electrophoresed on 1% gel [10].

## RESULTS

As the results show, the pAdCMV5-BFPq (7536bp) which contained lateral sites of E1 region (e.g. 0-1 and 9.4-15 map units of adenovirus) was digested to delete the previously cloned cassette region (2151bp) and make the construct (Figs .1 and 2).



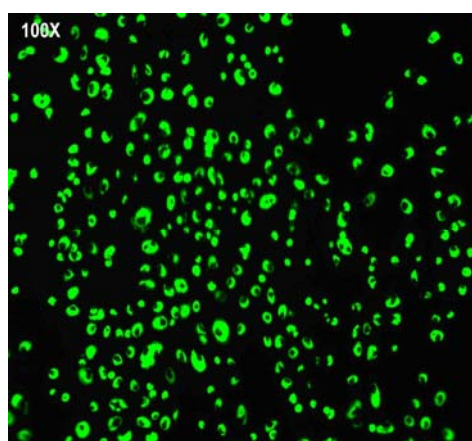
**Fig. 1.** Schematic representation of pAdCMV5-BFPq and construction of adenoviral plasmid. (A) Ad5-ITR, 217-550 (0-1 map unit on AdV5); Rabbit globulin poly A signal, 693-592; BFP from QBI, 1438-719; Enh MLP, 1631-1531; Adenovirus Tripartite leader, 1972-1768; CMV enhancer and promoter, 2502-1974; Ad5, 2731-5215 (9.4-15.5 map unit on Ad5); Ori, 5810-5610; B-Lactamase gene (Amp), 7330-6471. (B) Ad5-ITR, 217-550 (0-1 map unit on AdV5); Ad5, 580-3064 (9.4-15.5 map unit on Ad5); Ori, 3659-3459; B-Lactamase gene (Amp), 5179-4320. ITR, inverted terminal repeats; BFP, blue fluorescent protein; QBI, qbiogen; MLP, major late protein; CMV, cytomegalovirus.



**Fig. 2.** Agarose gel (1%) electrophoresis pattern of digested pAdCMV5-BFPq. The plasmid pAdCMV5-BFPq was digested to delete the previously cloned cassette and picked up the band 5385bp and ligated to construct adenoviral plasmid. Lane1, molecular size marker; Lane 2, digested pAdCMV5-BFPq.

Due to co-transfection of adenoviral DNA and adenoviral plasmid, the expected fluorescence recombinant E1 deleted AdV was obtained within 6 weeks from the starting point or 3 weeks after the production of the adenoviral plasmid (Fig. 3). Isolated plaques were eluted and three strongly positive fluorescent plaques selected for plaque purification.

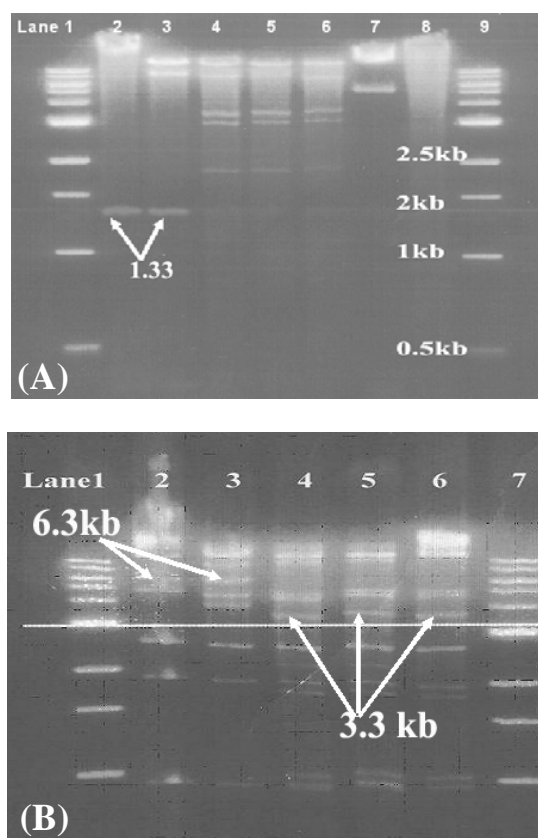
The generation of a pure viral stock necessitates 3 rounds of plaque purification and amplification that takes three months. Restriction fragments were analyzed to confirm the E1 deleted AdV (Fig. 4).



**Fig. 3.** Visualizing E1 deleted AdV by fluorescence imaging. The transfectants were selected on the basis of GFP expression by fluorescent microscope and three-well separated fluorescent plaques were picked up and used in plaque purification and adenoviral extraction process.

## DISCUSSION

Lately, AdV have made great gains in popularity due to their excellent potential for use as mammalian gene transfer vectors and especially in gene therapy related studies. The E1 deleted AdV has several advantages in compare to others: increasing transgene carrying capacity, decreasing the incidence of replication competent adenovirus generation in vector stocks, reducing or eliminating viral protein expression, particularly the cell function modulators which found in the E1 regions, adding additional blocks to viral replication in transduced tissues, and lowering the viral derived antigen burden [1, 2, 13, 14].



**Fig. 4.** Cut the DNA of E1 deleted AdV by restriction enzyme XbaI and NotI to confirm E1 deletion. To confirm the deletion of E1 gene in the AdV, the adenoviral DNA was cut by two different restriction enzymes. (A) XbaI: Lanes 1 and 9, molecular size markers; lanes 2 and 3, two different E1 positive adenoviral DNA (positive control); lanes 4-6; our designed E1 deleted AdV; Lane 7, an E1 negative adenoviral DNA (negative control) and Lane 8, uncut designed E1 deleted AdV. E1 deleted viruses and negative control did not have band of 1.33 kb. (B) NotI: Lanes 1 and 7, molecular size markers; Lanes 2 and 3 two different E1 positive adenoviral DNA (control) and lanes 4-6, our designed E1 deleted AdV. E1 positive viruses contained band of 6.3 kb but E1 deleted viruses did not have band of 3.3 kb.

The 293A cell line is a permanent line of primary human embryonic kidney cells transformed by sheared human adenovirus type5 (Ad5) DNA. This cell line contains the E1A and E1B, Ad5 viral genes that complement these genes deleted in the recombinant adenovirus.

In this project, adenoviral plasmid was transferred into the adenoviral genome by homologous recombination. The insertion of DNA by homologous recombination has enough efficiently to produce recombinant virus because of two reasons: 1) the adenoviral DNA is linear molecules and 2) the genome is too large (36 kb) for sufficient *in vivo* recombination. In conclusion Homologous recombination is more easy and fast technique in the production of AdV.

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