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GRT Golden Research Thoughts

PROMOTER DELINEATION OF *UL*-27 AND *UL*-28 OVERLAPPING CO-TERMINAL GENES OF BHV-1

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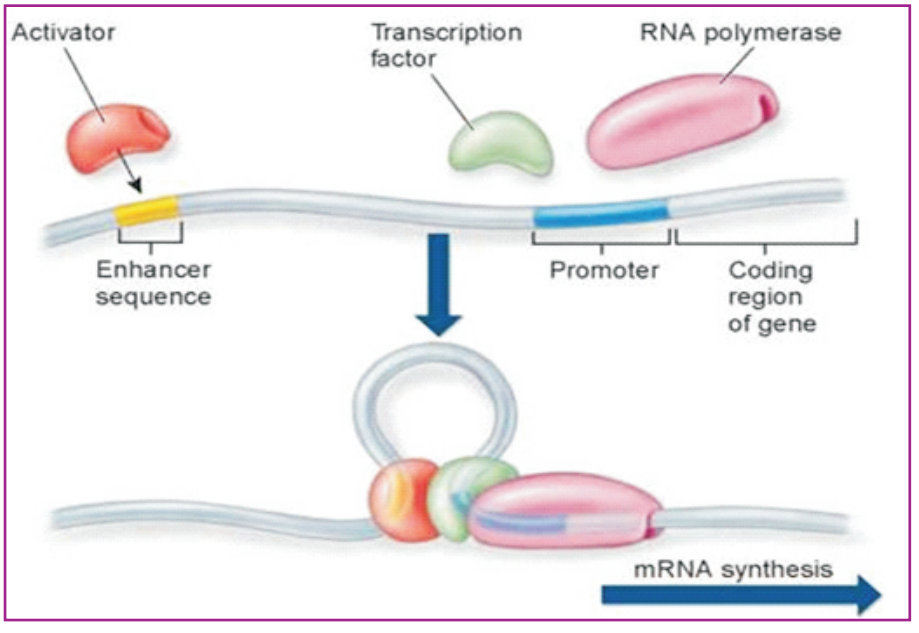

ABSTRACT

Bovine Herpesvirus type-1 (BHV-1) causes complex diseases like Infectious Bovine Rhinotrachitis (IBR), Infectious Bovine Vulvovaginitis (IPV) and mild conjunctivitis. BHV-1 is a member of alphaherpesvirinae sub-family with a genome of approximately 135.3 kbp. Ten of the twelve major envelope proteins (gB, gC, gD, gE, gG, gH, g1, gL, gM and gK) are glycosylated and two (gN and Us9) are non-glycosylated. Glycoprotein gB (UL-27) has a MW of 130 kDa and comprising of 932 amino acids essential for virus attachment, entry, cell to cell spread and fusion. Glycoprotein gB forms a complex with glycoprotein E. This complex has a role in cell to cell spread of virus. UL28 gene which encodes for DNA packaging terminase subunit 2 plays an important role in cleavage and encapsidation of viral DNA. UL28 and UL27 are co-terminal and overlapping genes. Through in-silico we had shown that both these genes have their own promoter located in the coding regions of upstream genes. The putative promoters for both the genes were cloned into promoter less vector and the promoter activity was measured. The promoter activity of UL28 gene which codes for DNA packaging terminase subunit 2 was much stronger than that of UL27 (gB) gene promoter. UL27 gene product gB is a major glycoprotein of the virus. Its low activity is surprise finding. Results indicate that UL28 and UL27 gene are transcribed through their own promoters.

KEYWORDS:*BHV-1, Promoter, overlapping genes, FACS*

INTRODUCTION

ORFs in the BHV-1 are oriented both in upstream and downstream directions. The sharing of nucleotides either on same strand or on different strand in same or opposite orientation for coding more than one protein as read in multiple reading

frames is called 'overlapping reading frames' or 'gene overlapping'. The plausible explanation for the phenomenon of gene overlapping in viruses assumed to be a form of genome compression which in turn physically limitsthe size of the capsid(Chirico*et al*.2010, Simon-Loriere*et al*. 2013, Brandes and Linial.2016).The general view is that the eukaryotic genes are transcribed by individual promoter in other words, majority of genes are mono-cistronic in nature. Reciprocally there are number of genes that uses multiple promoters having different tissue specificity and react differently to some signals. Although number of overlapping genes are rapidly expanding evolutionarily and great deal of information has been gathered through the years we still don't have the explanation forpromoter usage in overlapping genes.

The *UL*27 and *UL*28 genes are overlapping and co-terminal genes of BHV-1. How these genes are expressed is not known. They can express either from a single mRNA by scanning and leaky model or by other means. They can also express from their own promoters or common promoteris not known. In Varicella-Zoster Virus (VZV) glycoprotein gE and gI are transcribed both bi and monocistronically (Rahaus*et al.* 2003) while in BHV-1 gD and gI both the genes are expressed from their own promoters. In BHV-1 large numbers of genes arecoterminal with other genes. In one case four genes are coterminal (*UL*11, *UL*12, *UL*13, *UL*14) while in five cases two genes are coterminal (*UL*52&53, *UL*32&31, *UL* 27&28, *UL*24&25, *UL*6&7). In BHV-1 no studies has been made to identify the promoter usage in overlappingcoterminal genes except for gD and gI genes which are coterminalbutare non-overlapping(Chowdhury and Sharma, 2012). Hence for the present study we have taken a pair of overlapping co-terminal genesi.eUL-28 and *UL*-27.*UL*27 gene product is a major glycoprotein gB while *UL*28 gene product is a DNA packaging terminase subunit-2that are expressed in large amounts. The purpose of this study is to understand whether the *UL*28 and*UL*27 overlapping genes of BHV-1 use a common promoter or its individual promoter for gene expression.

MATERIALS AND METHODS

Virus, vector and cell line

Plaque purified Bovine Herpesvirus type-1 with the 10^9 units /ml was maintained at Immunochemistry Laboratory (ICL), Division of Biochemistry, Indian Veterinary Research Institute, Izatnagar 243 122, Bareilly, Uttar Pradesh, India.*Escherichia colistrain* DH5-α available at ICL was used for propagation of vectors.MDBK cell line was available at PPR Center, I.V.R.I., Mukteshwar. pDsRed Express N1 (4.7kb) expression vector was procured from B.D. Biosciences,USA.

In-silico promoter identification of UL-28 and UL-27 genes of BHV-1

The fulllength BHV-1genome (Ac. No. NC001847) was downloaded from NCBI website. Around 1000 nucleotides (nucleotide position: 52058-53058 for *UL*28 and 54394-55394 for *UL*27) upstream from the ORF start site (53058 for *UL*-28 and 55394 for *UL*-27) to their respective genes were selected for *In-silico* promoter prediction using online PROMOTER SCAN software tool. One set of primer was designed in Gene Tool software to amplify the predicted promoter regions of *UL*-28 and *UL*-27 gene. *Xho*I and *Eco*RI restriction sites were included in upper and lower primer respectively to ensure the orientation of insert in the vector.

DNA isolation from BHV-1 virus infected cells

Plaque purified BHV-1 virus was used to infect confluent MDBK cells. Following 18 hrs post- infection, when the cells were showing extensive cytopathic effect, the cells were freeze thawed three times to lyse the cells. The viral DNA from infectedcell lysate was extracted with proteinase K (250mg/ml) and with 5% SDS with incubation at 37°C for 15 mins and 60 mins respectively, followed by phenol-chloroform treatment. Concentration was checked in NanoDrop ND1000 Spectrophotometer and subsequently used as template for amplification of predicted promoter region from *UL*-28 and *UL*-27 genes in PCR.

PCR of putative UL-28 and UL-27 promoter

Standardization of PCR technique for *UL*-28 and *UL*-27 predicted promoter region was done individually using 50pmoles of each primer in a 50 µl reaction mixture containing 0.2 mol/ldNTPs, 0.5mM $MgSO_4$,Taq

polymerase buffer and approximately 25ng of purified viral DNA as a template. Then, 3U of Taq DNA polymerase was added and the reaction was carried out in a thermocycler for 35 cycles with an initial denaturation of 95°C for 5 min (cycling temperature of 95°C for 1 min, 53°C for 1 min, 72°C for 1 min). The final extension was carried out at 72°C for 10 min.

The PCR products were visualized by Ethidium bromide staining in 1.0% agarose gel electrophoresis and approximate size was calculated from the standard DNA molecular weight markers. The amplified PCR products were purified using HiperTM Mini spin gel extraction Kit (Himedia). Finally, the DNA was eluted by centrifugation at 13,000 rpm for 2 min.

The binding sites of primers and their size of amplified PCR productwere shown in Table-1.

Cloning and characterization of putativeUL-28 and UL-27 specific promoter

The gel purified PCR products UL-28 and UL-27 were cut with *Xho*I and *Eco*RI, and inserts were ligated discretely into pDsRed-revCMV vector precut with the same enzymes. The ligated DNA was transformed in E-coli DH5aa cells and plated on agar plates containing 50µg/µl Ampicillin/Kanamycin. In frame cloning and orientation of insert of UL-28 and UL-27 promoter specific clones were verified by asymmetric restriction site digestion using *Xho*I and EcoRIenzymes and DNA sequencing of plasmid-insert junctions. The confirmed promoter specific clones were made endotoxin free by Midiprep Purification Spin Kit (Himedia). The elute containing endotoxin free plasmid and its concentration was checked in NanoDropND1000 Spectrophotometer. This endotoxin free plasmid used for transfection study in Verocell line as described.

Transfection study in Vero cells

Vero cells were grown in 6 well plates up to 70% confluence in Dulbecco Modified Essential Media (DMEM) with 10% serum. Old media was discarded and the cells washed with 2 ml of prewarmed DMEM media. In a micro centrifuge tube, 60 µl of Escort transfection Buffer (Sigma) and 9µl of Escort transfection reagents were taken and mixed properly. In another tube, 20 µl of plasmid DNA with or without insert (5µg) was mixed properly with 60µl of Escorttransfection buffer. Content of both the tube were mixed and incubated at room temperature for 20 minutes. The transfection cocktail was mixed and layered over the monolayer and incubated at 37°C in 5% CO_2 incubator (Nuire) for 48 hr.

FACS analysis

Media from transfected wells were discarded and cells washed with prewarmed (37°C) PBS (pH7.2). Later, 50 µl of prewarmed TVG was added and monolayer was dislodged into single cell. The activity of TVG was inactivated by adding 300 µl of chilled PBS and cells were pelleted by centrifugation at 3,000 rpm for 5 min. The pellet was dispersed in 250 µl of PBS and analyzed in FACS (Flouroscent Assisted Cell Sorter) (BD Dickinson, USA) in FL2 channel.

RESULTS AND DISCUSSION

DNA isolation from BHV-1 virus infected cells: Following infection of MDBK cells (Fig 1a) with Plaque purified BHV-1 virus a characteristic cytopathic effect was observed 18 hrs post- infection (Fig 1b).

Promoter prediction and amplification: PROMOTER SCAN analysis of selected regions(52058-53058 and 54394-55394) upstream to*UL*28 and *UL*27ORF'spredicted a putative promoter at their respective nucleotide positions 52652-52902 and 55116-55366.These *UL*28 and *UL*27 predicted promoters were present in the coding region of *UL*29 and *UL*28generespectively. Total genomic DNA isolated from BHV-1infected MDBK cellsyielded a clear band inagarose gel (Fig 2A).The concentration of DNA extracted from infected cell lysate was 100ng/µl. The primers designed for *UL*28 gave an amplified product of about 357 bp as expected (Fig 2B) while primers for *UL*27 gave an amplification product of 373 bp product in PCR (Fig 2D). The amplification was obtained only when 1 µl of DMSO was added to 20 µl reaction mix as BHV-1 genome contains more than 70 % GC bases which requires co-solvents for amplification.

Cloning and characterization of recombinant plasmids: Predicted promoter regions were amplified and

cloned into pDsRedCMVrevvector. CMV sequence of pDsRed Express N1 was cloned in reverse orientation in same vector to design a promoterless vector (pDsRedCMVrev) in Immunochemistry Laboratory (ICL), Division of Biochemistry, Indian Veterinary Research Institute, Izatnagar 243 122, Bareilly, Uttar Pradesh, India (Chowdhury and Sharma, 2012). The CMV sequence in opposite direction also acts as enhancer (Schmidt *et al*. 1990).

The *Xho*I and *Eco*RI digested PCR products (*UL28 and UL-27*) were ligated individually into promoterless vector pDsRedCMVrevvector precut with the same enzymes. The recombinant clones, containing *UL*28 and *UL*27 released an insert size of 357bp(Fig2C) and 373bp (Fig 2E) respectively when digested with *Xho*I and *Eco*RI indicating presence of target sequence in the vector. Thus the recombinant plasmids were designated as pDsRedCMVrev-UL28 and pDsRedCMVrev-UL27. These plasmids were further confirmed by custom sequencing. The sequence matched with reported sequence of BHV-1 virus (Sequence not shown). The concentration of endotoxin free plasmids purified from 50 ml of LB broth was 300ng/µl as measured spectrophotometrically.

Analysis of promoter activity in FACS: The constructs were transfected in Vero cells and fluorescence of the Red fluorescent protein (RFP) was studied in FL-2 channel of flow cytometer (FACS Callibur)from the cloned *UL*28 and *UL*27promoter in pDsRedCMVrev vector are shown in Fig 3. *UL*28 putative promoter expressed the reporter gene at higher level than *UL*27 promoter but at level considerably lower than CMV promoter (wild type) Fig 4.The *UL*27 gene product gBis a major glycoprotein indicating a strong promoter for its gene which is differing to our anticipation.This may be due to the fact thatpromoter activity depends on large number of factors which influence its activity both *in-vitro* as well as *in-vivo*. Hence we tried to explain the rationale of low promoter activity of a major glycoprotein gB*in-vitro.*

Long range interactions: The transcriptional regulation in BHV-1 is highly coordinated as defined in Multigene(MG)complex model where large number of genes from control regions with the same genomic span interact each other (Kuznetsova*et al.* 2015). In the multigene complex, RNAPII binding strengthens promoter - promoter interactions of different expression kinetic classes (alpha, beta, gamma-1 and gamma-2) and also promoter interacts with distant acting enhancers providing an ideal topological framework for potential transcription coordination, thereby facilitating efficient expression of overlapping viral genes such as *UL*-28 and *UL*-27 of BHV-1 *in-vivo*(Gong *et al*.2011). But in our study we cloned the promoters of *UL*-28 and *UL*-27 individually without distal enhancers which might be present few kilobases away in the viral genome(Tena*et al*. 2014). For the reason, predicted promoter sequence of *UL*-27 displayslow promoter activity like basal promoter region.

Intrinsic enhancer like activity of CMV promoter: The CMV sequence of pDsRed Express N1 was cloned in reverse orientation in same vector to design aspromoterless vector where the CMV sequence in opposite direction acts as enhancer (Boshart*et al*.1985, Schmidt *et al.* 1990). Although the common property of promoters with enhancer capacity that could influence other promoters, intriguingly there is an inverse relationship between enhancer and promoter function. In other words, stronger promoters conveyed trivial enhancer functions and vice versa as observed by Kuznetsova*et al.* (2015).

*Genomic distance:*The general view is that there is a pre-established long-range interactions between promoters and distant acting enhancers via transcriptional looping that connect the target genes of different kinetic classes (alpha, beta, gamma) distributed randomly over hundreds of kilobases away on genomic length(Kuznetsova*et al*. 2015).The close genomic distance between predicted promoter and CMV enhancer-like sequence in our construct correlates with the reported findings that more than 40% of the enhancers do not interact with their nearest promotersexplaining the low promoter activity of major glycoprotein gBin-vitro (Li*et al.* 2014). The genomic distance and intrinsic enhancer like activity of CMV promoter in the promoterless construct needs further investigation.

Through *in-silico* analysis and reporter gene assay, we predicted putative promoter regions for both *UL*27 and *UL*28 overlapping coterminal genes of BHV-1.Our most interesting finding was regardless of considerable geneoverlapping and of different/same kinetic class both the genes comprise its own regulatory promoter regions .

CONCLUSION

The experimental evidence from reporter gene assay enlarged our limit of identifying promoter boundary to remote enhancers that are embedded in the distantly located genes (Visel*et al*. 2009) as this promoter-enhancer interactions are crucial for efficient expression and coordinated transcriptional regulation of related genes in BHV-1. Also the genomic distance and intrinsic enhancer like activity of CMV promoter in the promoterless construct needs further investigation.

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REFERENCES

1.Boshart M, Weber F, Jahn G, Dorsch-Hasler K, Fleckenstein B andSchaffner W. 1985. A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus.Cell41(2):521-30

2.Brandes N and Linial M. 2016. Gene overlapping and size constraints in the viral world. Biology Direct 11:26

3.Chirico N, VianelliAandBelshaw R. 2010.Why genes overlap in viruses. Proceedings of the Royal SocietyB 277(1701):3809-17

4.Chowdhury S and Sharma B. 2012. Transcription of gD and gI genes in BHV1-infected cells. Journal of Biosciences37 (6):971-7.

5.Kuznetsova T, Wang S Y, Rao N A, Mandoli A, Martens J H, Rother N, Aartse A, Groh L, Janssen-Megens E M, Li G, Ruan Y, LogieCandStunnenberg H G. 2015. Glucocorticoid receptor and nuclear factor kappa-b affect three-dimensional chromatin organization. Genome Biology16:264

6.Li G, Cai L, Chang H, Hong P, Zhou Q, Kulakova E V, Kolchanov N A andRuan Y. 2014. Chromatin Interaction Analysis with Paired-End Tag (ChIA-PET) sequencing technology and application. BMC Genomics15:11

7.Rahaus M, Desloges N, Yang M, Ruyechan W T and WolffM H. 2003. Transcription factor USF, expressed during the entire phase of varicella- zoster virus infection, interacts physically with the major viral transactivator IE62 and plays a significant role in virus replication.Journal of General Virology84:2957-67

8.Gong F, Sun L, Wang Z, Shi J, Li W. 2011. The bcl2 gene is regulated by a special at-rich sequence binding protein 1-mediated long range chromosomal interaction between the promoter and the distal element located within the 3-utr. Nucleic acids research39: 4640–4652

9.Schmidt E V, Christoph G, Zeller R and Leder P. 1990. The cytomegalovirus enhancer: a pan-active control element in transgenic mice. Molecular and Cellular Biology10 (8):4406-11

10.Simon-Loriere E, Holmes E C and Pagan I. 2013. The effect of gene overlapping on the rate of RNA virus evolution.Molecular Biology and Evolution30(8):1916-28

11.Tena J J, Gonzalez-Aguilera C, Fernandez-Minan A, Vazquez-Marin J, Parra-Acero H, Cross J W, Rigby P W, Carvajal J J, Wittbrodt J, Gomez-SkarmetaJLandMartínez-Morales J R. 2014. Comparative epigenomics in distantly related teleost species identifies conserved cis-regulatory nodes active during the vertebrate phylotypic period. Genome Research24(7):1075-85

12.Visel A, Rubin E M and Pennacchio L A. 2009.Genomic views of distant-acting enhancers. Nature461 (7261):199-205.

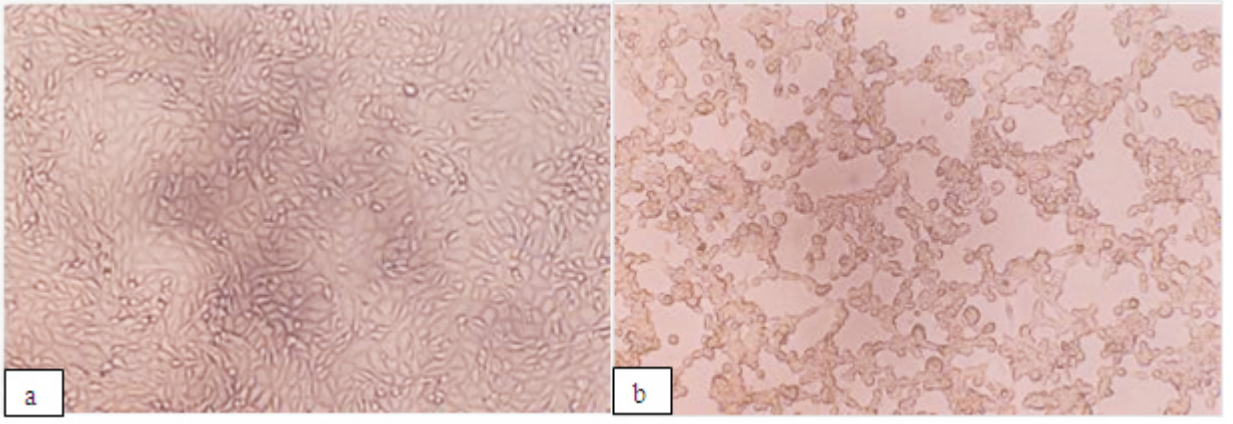

Fig.1. (a) Monolayer of MDBK cell line(b) CPE in BHV-1infected MDBK cell at 18 hrs Post infection

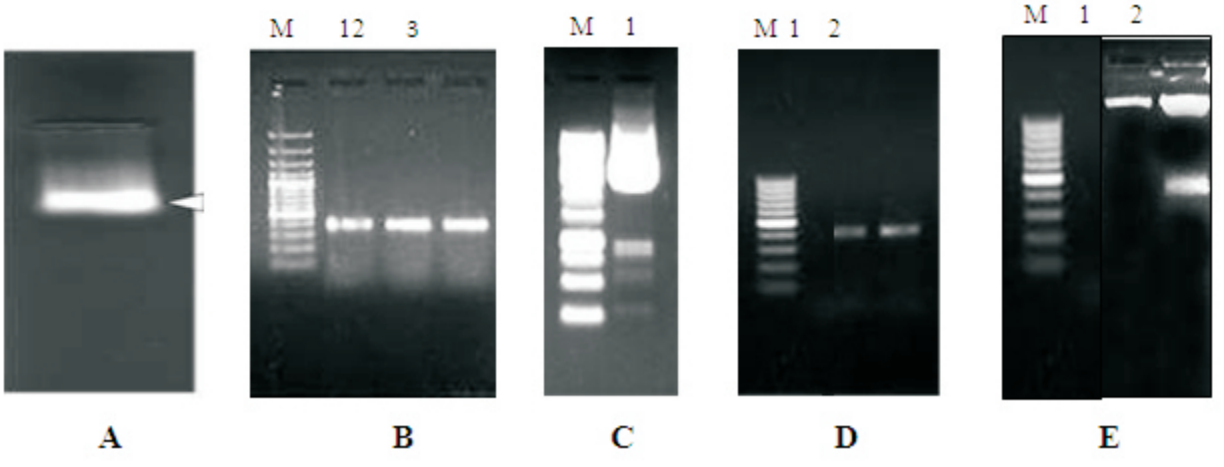

Fig.2. Genomic DNA isolation, PCR amplification , cloning and characterization of recombinant Plasmid; A. Genomic DNA isolated from BHV-1 infected MDBK cells B. Amplification of Putative UL28 Promoter (Lane 1 to 3 – amplicon size of 357 bps) C. 357 bp insert release by EcoR1 and Xho1 from pDsRed ExpressN1 D. Amplification of Putative UL27 Promoter (Lane 1&2 - amplicon size of 373 bp bps) E. 373 bp insert release by EcoR1 and Xho1 from pDsRed Express N1and Lane M - 100bp DNA ladder Gene ruler, Fermentas).

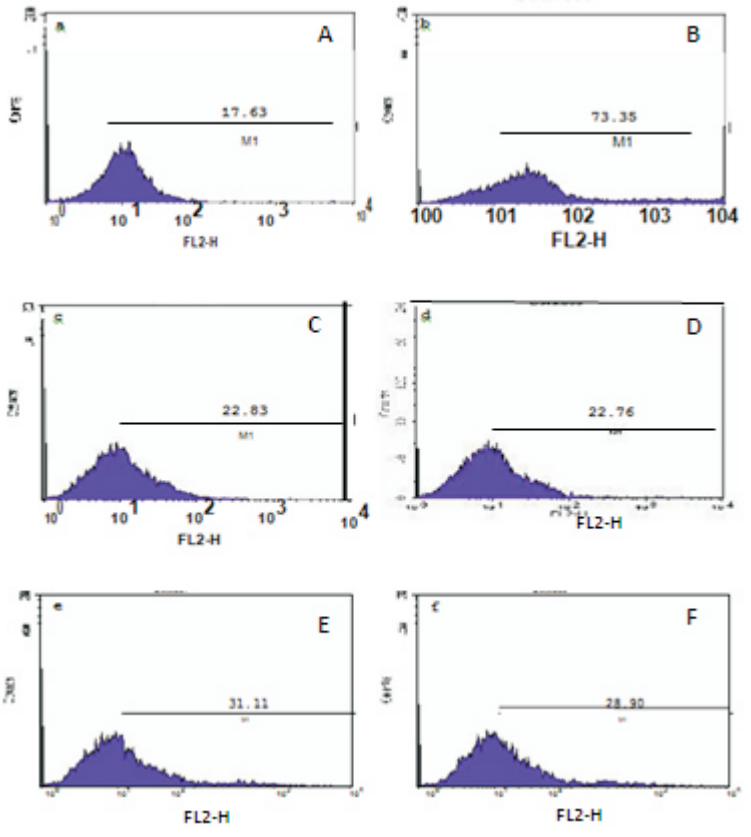

Fig.3.FACS analysis of delineated UL27 and UL28 Promoters in Vero cell lines
(A) - Vero cell control (B) – wildtypepDsRedExpressN1 vector (C) and (D) - pDsRed rev CMV delineated UL27 promoter clone (E) and (F)-pDsRed rev CMV delineated UL28 promoter clone

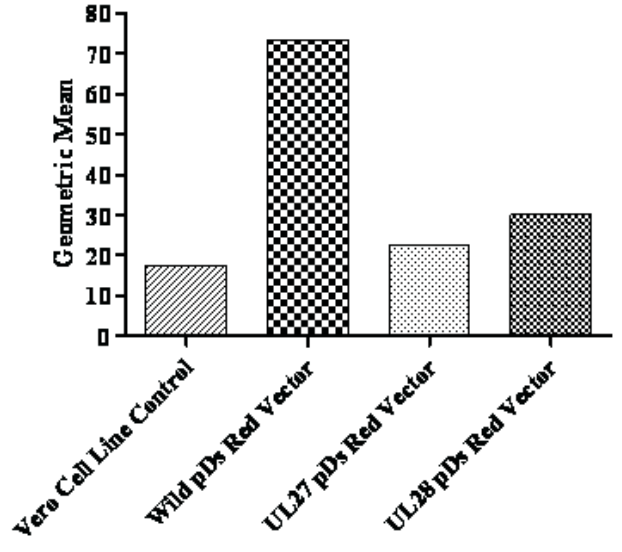

Fig.4. RFP expression of UL27 and UL28 Putative Promoter in FACS the expression of the reporter gene (RFP) in FL-2 channel by UL-28 putative promoter was higher than UL-27 promoter but at level considerably lower than CMV promoter (wild type).

Table1.Primers for Putative Promoters (UL-27 and UL-28) were designed using Gene tool software programme

Primers	Primer sequences	Primer Binding position	Annealing Temp (^{0}C)	PCR product size (bp)
UL-28P Forward	5' CGGCTCGAGGGATGGTCGCCGGAGGG 3'	52588 - 52607	53	357
UL-28P Reverse	5' TTGAATTCGCGGCAAACGGCGCATGATTA 3'	52926- 52945	53	
UL-27P Forward	5' CGCTCGAGGACGGCAGCGCGCGCACAACTTC 3'	55031 -55051	60	373
UL-27P Reverse	5' GCGAATTCAAGCGGCCATGACTCCTCGTAG 3'	55383 - 55403	59	

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