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# Title: Interactions *in-vivo* between the Vif protein of HIV-1 and the Precursor ($Pr55^{GAG}$) of the Virion Nucleocapsid Proteins.

**Running Title: Vif - $Pr55^{GAG}$ interactions in HIV-1**

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**Abstract = 177 words**

**Main Text = 2334 words**

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# Abstract

The abnormality of viral core structure seen in *vif*-defective HIV-1 grown in PBMCs has suggested a role for Vif in viral morphogenesis. Using an *in-vivo* mammalian two hybrid assay the interaction between Vif and the precursor ($Pr55^{GAG}$) of the virion nucleocapsid proteins has been analysed. This revealed the amino terminal (aa 1-22) and central (aa 70-100) regions of Vif to be essential for its interaction with $Pr55^{GAG}$ but deletion of the carboxy terminal (aa 158-192) region of the protein had only a minor effect on its interaction. Initial deletion studies carried out on $Pr55^{GAG}$ showed that a 35 amino acid region of the protein bridging the MA(p17)-CA(p24) junction was essential for its ability to interact with Vif. Site directed mutagenesis of a conserved tryptophan ($Trp^{21}$) near the amino terminus of Vif showed it to be important for the interaction with $Pr55^{GAG}$. By contrast mutagenesis of the highly conserved YLAL residues forming part of the BC-box motif, shown to be important in Vif promoting degradation of APOBEC3G/3F, had little or no effect on the Vif - $Pr55^{GAG}$ interaction.

# Introduction

In addition to the canonical gag, pol and env genes found in all retroviruses, the human immunodeficiency virus (HIV-1) in common with most other lentiviruses encodes six regulatory proteins (Tat, Rev, Vpr, Vif, Nef and Vpu) usually referred to as accessory proteins. Of these Vif is encoded by all lentiviruses with the exception of equine infectious anaemia virus (EIAV) [29] and in several animal models it has been shown to be essential for virus infection [10, 17]. In HIV-1, Vif is a 23KDa basic protein that is required in a cell type dependent fashion for the production of infectious virus [21]. Thus most $CD4^+$ continuous cell lines give normal virus yields when infected with vif deleted virus and are consequently said to be permissive, whereas others such as H9 cells and crucially PBMCs, the normal *in-vivo* host cells for HIV, give greatly reduced virus yields of Vif minus virus and are termed non-permissive or restrictive [12, 14, 15]. Studies on heterokaryons made between permissive and restrictive cells showed that the restrictive phenotype was dominant indicating the presence in restrictive cells of an inhibitor of virus replication [25, 34]. In an elegant study this inhibitor was identified as a member of the APOBEC family of cytidine deaminases [32]. A series of subsequent studies have shown that in the absence of Vif, APOBEC3G/3F expressed in restrictive cells is packaged into virus particles and leads to hypermutation of the viral genome following deamination of cytidine to uridine (~~C~~ U) during synthesis of minus strand viral cDNA [18, 26, 40, 41]. Despite the recent focus on the role of Vif in blocking the action of APOBEC, earlier studies on Vif deleted virus produced from restrictive cell lines revealed the virions to have malformed viral core structures pointing to a possible role for Vif in modulating the processing by the viral protease of the $Pr55^{GAG}$ precursor that leads to the formation of the viral nucleocapsid [4, 6, 19]. However attempts to demonstrate a direct role for Vif in the morphogenesis of virus particles has produced conflicting results [5, 13]. In a previous study examining the sequence of the vif gene in virus isolated from patients undergoing treatment with antiviral drugs directed at the viral protease we were able to show a linkage between specific amino acids at a number of positions in Vif and the development of resistance to protease inhibitors [1]. These data are again suggestive of a role for Vif in modulating the action of protease in processing of the $Pr55^{GAG}$ precursor. In the present study we have made use of a mammalian two-hybrid assay [31, 37] to screen for direct interactions *in-vivo* between Vif and both the viral protease and its substrate $Pr55^{GAG}$.

# Materials and Methods

(i) **Plasmids:** The pM and pVP16 mammalian two-hybrid vectors from the 'Matchmaker™' system (Clontech) were used to generate fusion protein constructs for use in two-hybrid assays. Two reporter plasmids were employed, pG5CAT (Clontech) which expresses Chloramphenicol acetyl transferase (CAT) under the control of a Gal4 responsive promoter and pUAST-hrGFP-neo which expresses green fluorescent protein (GFP) also under the control of a Gal4 responsive promoter. The latter plasmid was obtained from Dr K.T. Chung (University of Warwick).

The vif sequences used in constructing two hybrid fusion vectors were generated by PCR from a cDNA sub-clone carrying the vif gene generated from the HXB2 isolate of HIV-1 [7]. All cDNA clones whose derivation involved PCR were re-sequenced to ensure that no adventitious mutations had been introduced during PCR amplification. The coding sequence for $Pr55^{GAG}$ was obtained by sub-cloning from a cDNA clone of the BH-10 isolate of HIV-1 [11] and the coding sequence for the viral protease was also obtained by sub-cloning from a BH-10 derived DNA clone [2].

(ii) **Propagation and Transfection of Mammalian Cells:** The COS-1 line of African green monkey kidney cells [16] was used for all two hybrid assays. The cells were grown in Glasgow modified MEM supplemented with 10% foetal calf serum. For use in transfection experiments cells were plated in 12-well tissue culture dishes and used at ~80% confluency. Each well of cells was transfected with a mixture containing 1µgm of each of the three plasmids and 3.5µl of Lipofectaine 2000 (Invitrogen). In all experiments cells were transfected in parallel with a plasmid expressing GFP constitutively from the immediate early promoter of cytomegalovirus to allow transfection efficiency to be measured so that comparability between experiments could be ensured.

(iii) **Mammalian Two-Hybrid Assays:** Two reporter genes were employed in this study. GFP has the advantage that it can be simply assayed by UV microscopy of live cells but its limitation is that it gives a largely qualitative plus-minus result. By contrast the CAT reporter requires the preparation of cell extracts and therefore assaying it is more labour intensive but it has the advantage of giving a quantitative measure of reporter gene expression. Conseqently

in most cases interaction assays were initially scored using the GFP reporter and then the strength of the interaction assess using the CAT reporter.

GFP expression was assayed in live cells by examining them under a UV microscope at 72 hours after transfection. CAT reporter expression was assayed in cytoplasmic extracts of transfected cells also made at 72 hours after transfection using a ELISA based CAT assay kit from Roche as described in the manufacturer's instructions.

(iv) **GST Pulldown Assays:** GST-Vif and GST- $Pr55^{GAG}$ fusions used as 'bait' proteins in the assay were constructed by insertion of the relevant open reading frames into the pET42b vector (Novagen). After overnight induction of bait protein expression in E.coli (BLR) with 1mM IPTG, cells with lysed by French press and a 30,000g supernatant containing the soluble GST fusions prepared for binding to glutathione agarose beads for six hours at $4^0$C. After washing extensively with phosphate buffered saline (PBS) to remove unbound proteins the beads were used in binding assays. Radio-labelled test proteins were prepared using the TNT Quick (Promega) coupled transcription-translation system according to the manufacturers instructions. In all cases the system was primed with a plasmid carrying the ORF of the test protein inserted downstream of the T7 promoter. 100,000cpm of each test protein was incubated with either of the two bait proteins bound to glutathione beads in PBS-1% Triton-X-100 for six hours at $4^0$C. After extensive washing with PBS-1% Triton-X-100 the bound protein was eluted by boiling in 2% SDS-5% β-ME and the retained radioactivity measured by liquid scintillation counting.

(v) **Mutagenesis:** Deletion and site directed mutagenesis of the pVP16-Vif and pM- $Pr55^{GAG}$ fusion vectors were both carried out using PCR based techniques as previously described [20, 30].

# Results

(i) **Screening for *in-vivo* interactions between Vif and Pr55$^{GAG}$ or Protease (PR).**

The GFP reporter was employed to provide a rapid and convenient assay for screening live cells for evidence of interaction between two-hybrid fusion constructs co-transfected into COS-1 cells. A clear interaction between Vif and Pr55$^{GAG}$ was detected with fusion constructs of either protein to both the Gal4 DNA binding domain and the VP16 activation domain (Fig 1). By contrast no evidence of interaction between Vif and PR was seen with either of the PR fusion constructs generated (Fig1 and results not shown). PR also failed to show any interaction with Pr55$^{GAG}$ (Fig 1).

The clear interaction between Vif and Pr55$^{GAG}$ was confirmed in cell free extracts made from cells co-transfected with the CAT reporter which allowed a more quantitative assessment of the interaction (Fig 2).

(ii) **Mapping the regions of Vif interacting with Pr55$^{GAG}$.**

Initial attempts to localise the regions of Vif involved in the interaction with Pr55$^{GAG}$ made use of three deletion mutants of Vif covering the amino and carboxy terminal regions of the protein and a central region encompassing amino acids 70-100 (see Fig 3). Two hybrid assays carried out using these mutants revealed that both the amino terminal 26 amino acids and the central region of the protein were essential for the interaction whereas loss of the carboxy terminal 35 amino acids only resulted in an approximately 40% drop in reporter gene expression which nevertheless remained clearly positive in comparison to the negative controls. (Fig 4A). The results shown in this and all other figures have been repeated on at least three occasions and a complete set of illustrative results is shown in each case

In a previous study on Vif we have reported on the importance of the conserved tryptophan (Trp) at amino acid 21 to the functioning of Vif to generate infectious virus when propagated in restrictive cell lines [7]. It was therefore of interest to examine the effect on Vif's interaction with Pr55$^{GAG}$ of introducing the same mutational changes at amino acid 21 as those examined in the earlier studies. When this was done there was a striking correspondence between results

obtained in the two hybrid assay and those found in the earlier study, in that changing the $Trp^{21}$ to arginine, isoleucine or tyrosine resulted in a greater than 80% drop in reporter gene expression whereas substitution with phenyalanine gave wild levels of reporter gene activity (Fig 4B).

In an attempt to localise other regions of Vif that might contribute to its interaction with $Pr55^{GAG}$ attention was focused on the conserved BC-box motif that has been shown to be crucial to the interaction of Vif with the Cul5-EloBC complex required to induce the ubiquitin-proteasome based degradation of the APOBEC3G/3F viral inhibitor [28, 39]. Site directed mutagenesis was employed to make single and double amino acid changes in the highly conserved YLAL region of this motif. However when the mutated Vif was used in the two hybrid assay there was at most an ~40% reduction in reporter gene expression (Fig 4C) indicating that this highly conserved motif is not essential for the Vif-$Pr55^{GAG}$ interaction.

**(iii) Mapping the regions of $Pr55^{GAG}$ interacting with Vif:**

To begin the process of localising the region(s) of $Pr55^{GAG}$ involved in interacting with Vif a number of deletion mutants of the protein were constructed. These were focused primarily on the cleavage sites in the protein for the viral protease (see Fig 3). The results obtained when these mutants were used in two hybrid assays are shown in Table 1. Deletion of the either the amino terminal or carboxy terminal 35 amino acids, deletion of L1 or deletion of L1 and SP1 from the carboxy terminus of the protein all had no effect on reporter gene expression. By comparison if either the coding sequence for MA or a 35 amino acid region encompassing the MA-CA junction were deleted then reporter gene expression was reduced by >80% (Fig 5) indicating the importance of this region of $Pr55^{GAG}$ to the interaction with Vif.

**(iv)Validation of mammalian results using in-vitro GST Pull-down assay:**

To validate the protein interactions seen in-vivo with the mammalian two hybrid assay GST pull-down assay was used. The results obtained with this in-vitro assay (Fig 6) confirmed those obtained in-vivo, with wild type Vif and the Vif mutant with Phe replacing the Trp at position 21 both giving a clear interaction with $Pr55^{GAG}$. By contrast the Tyr21 substitution of Vif caused the loss of $Pr55^{GAG}$ interaction (Fig 6). The NSP1 protein of rotavirus which is

an RNA binding protein also failed to interact with $Pr55^{GAG}$ in this assay (Fig 6) making it unlikely that the observed Vif- $Pr55^{GAG}$ interaction involved an RNA intermediate.

## Discussion

The purpose of the present study was to extend a range of earlier studies [4-6, 13, 19] including our own [1] which have focused on a role for Vif in the process of viral morphogenesis. The mammalian two hybrid assay used in this study provided clear evidence of an interaction between Vif and $Pr55^{GAG}$ *in-vivo*. This result is in line with an earlier, primarily *in-vitro*, study using a GST pull down assay [5]. By contrast no evidence of any interaction occurring between Vif and the protease of HIV-1 was found in the mammalian two hybrid assay which contradicts results obtained using ELISA assays with HIV proteins expressed in and purified from *E.coli* [3]. The negative result obtained in the mammalian two hybrid assay does however need to be treated with some caution as attempts to show an interaction between the viral protease and its substrate ($Pr55^{GAG}$), which must occur if only transiently during virion assembly, were also unsuccessful.

The initial deletion analysis aimed at localising the region(s) of Vif involved in the interaction with $Pr55^{GAG}$ showed that the amino terminal region and a central area of Vif were both important for this interaction. By contrast deletion of the carboxy terminal 35 amino acids of Vif produced only a relatively small drop (~40%) in the level of interaction with $Pr55^{GAG}$ as measured by reporter gene expression in the mammalian two hybrid assay. This last result is at odds with earlier results using the *in-vitro* GST pull-down assay where deletion of the carboxy terminal 22 amino acids of Vif abolished its interaction with $Pr55^{GAG}$ [5]. It seems probable that this discrepancy between the two studies can be attributed either to differences in the assay protocol (*in-vivo* vs *in-vitro*) and/or differences in the nature of the fusion partner employed (VP16 and Gal4 DNA binding domain vs Glutathione S transferase) and the effects that this may have on the properties of the fusion protein.

The initial deletion studies carried out on $Pr55^{GAG}$ showed that removal of 35 amino acids at the junction of MA (p17) and CA (p24) was sufficient to completely abolish the interaction with Vif pointing to the main site of interaction lying in this region of the protein. This result is partially consistent with that found in the earlier *in-vitro* study [5] where the MA-CA junction was also identified as a region involved in interacting with Vif. However the earlier study also identified a second site of interaction in the NC (p7) region of $Pr55^{GAG}$ that was not found in the current study [5]. Again this difference may be due to the detailed differences in both the

constructs and assay protocols employed and should not at this stage be taken to indicate the presence of only one interacting site between the two proteins *in-vivo*.

Site directed mutagenesis of Vif to more specifically localise regions of the protein involved in the interaction with $Pr55^{GAG}$ revealed that the conserved tryptophan at position 21 was important. Change of this conserved position to arginine, isoleucine or tyrosine resulting in a greater than 80% drop in reporter gene expression in the two hybrid assay. By contrast substitution of the tryptophan with phenylalanine had no effect on the Vif - $Pr55^{GAG}$ interaction. These in-vivo results were corroborated by similar findings in an in-vitro GST pull down assay. This is interesting in the context of our earlier work showing that these same mutational changes when carried out on an infectious DNA clone of HIV resulted in the same effects on the biological phenotype of virus produced in non-permissive cells [7]. That is substitution with arginine, isoleucine or tyrosine resulted in a Vif –minus phenotype whereas substitution with phenylalanine gave a wild type Vif phenotype [7]. More recent studies aimed at mapping the regions of Vif involved in overcoming the APOBEC3G/3F based inhibition of HIV replication have confirmed the importance of this conserved tryptophan [36]. This study further argued that $Trp^{21}$ mediated its effect on the APOBEC3G/3F inhibition by affecting the Vif directed degradation of APOBEC3G/3F, although no data on degradation were presented for this specific tryptophan [36]. The results obtained on mutagenesis of the highly conserved YLAL residues that form part of the BC-box motif found towards the carboxy terminus of Vif were of interest in the context of the known involvement of this motif in APOBEC3G/3F degradation [28, 39]. Despite it having been shown to be crucial for the formation of the complex with Cul5-EloBC involved in targeting APOBEC3G/3F for proteasomal based degradation [28, 39], both single and double amino acid changes in the YLAL sequence had little or no effect on the Vif - $Pr55^{GAG}$ interaction.

It has recently been shown that in the absence of functional Vif, APOBEC3G/3F will be incorporated into assembling viral capsids by interacting with the amino terminal region of NC in $Pr55^{GAG}$ [8, 24]. The confirmation in the present study that Vif also interacts *in-vivo* with $Pr55^{GAG}$ raises the speculative possibility that in addition to its role in promoting the degradation of APOBEC3G/3F in non-permissive cells [9, 22, 23, 27, 33, 35, 38] Vif may also directly compete with APOBEC3G/3F for incorporation into the assembling virus particle. If further studies aimed at mapping more precisely the binding sites for Vif on $Pr55^{GAG}$ are able to confirm the earlier *in-vitro* studies indicating that both proteins have interaction sites that

involve the NC region of $Pr55^{GAG}$ [5, 8, 24], then it will be important to establish whether or not it is the effects that Vif may have on the incorporation of APOBEC3G/3F into assembling virions that represent its primary route to relieving the APOBEC based block to viral replication in normal host cells for HIV.

# Figure Legends

**Fig 1: Analysis of Interactions between Vif, Protease and $Pr55^{GAG}$ using a GFP reporter in Mammalian Two-Hybrid Assays**

COS-1 cells (5 x $10^4$ cells/well) were plated out in twelve well tissue culture plates to give ~80% confluent monolayers after overnight incubation at $37^0$C. These cells were co-transfected with the Gal4 responsive GFP reporter ( pUAST-hrGFP-neo) plasmid and pM (Gal4 DNA binding domain) and pVP16 (Gal4 Activation domain) fusion constructs with Vif, Protease and $Pr55^{GAG}$ as described in Materials and Methods. A positive control transfection in which cells were co-transfected with reporter plasmid and fusion constructs of p53 and large T from SV40 which are known to interact in mammalian cells was included in all assays. The cell monolayers were examined by conventional light and UV microscopy three days after transfection and scored for reporter gene expression. Each panel of the figure consists of a pair of images, the left hand of which shows the cell monolayer viewed using conventional white light and the right hand of which shows reporter GFP expression in the same field viewed under UV light. The yellow size bar shown in panel A is 200nm and is provided to give an indication of magnification used in all panels.

Panel A: Interaction between an activation domain fusion of Vif (AD-Vif) and a DNA binding domain fusion of $Pr55^{GAG}$ (BD- $Pr55^{GAG}$).

Panel B: Interaction between a DNA Binding domain fusion of Vif (BD-Vif) and an activation domain fusion of $Pr55^{GAG}$ (AD- $Pr55^{GAG}$).

Panel C: Interaction between an activation domain fusion of Vif (Ad-Vif) and a DNA binding domain fusion of Protease (BD-Protease).

Panel D: Interaction between an activation domain fusion of $Pr55^{GAG}$ (AD- $Pr55^{GAG}$) and a DNA binding domain fusion of Protease (BD-Protease).

Panel E: Positive control showing interaction between p53 and the large T antigen of SV40 virus.

**Fig 2: Analysis of the Interaction between Vif and $Pr55^{GAG}$ using a CAT reporter in the Mammalian Two-Hybrid Assay.**

COS-1 cells (~80% confluent) were co-transfected with the Gal4 responsive CAT reporter (pG5CAT) and various Vif and $Pr55^{GAG}$ fusion constructs as indicated under each column of the bar chart using the construct abbreviations given in Figure 3. At three days post transfection the cell monolayers were harvested and the level of reporter CAT expression assayed in cell free extracts using a CAT ELISA assay as described in Materials and Methods. The positive control used in these assays was co-transfection with fusion constructs expressing the interacting partners p53 and large T of SV40 virus. The negative control was co-transfection of cells with the two interaction plasmids carrying no fusion inserts.

**Fig 3: Schematic Diagram to show the Mutational Analysis of Vif and $Pr55^{GAG}$ employed to map interacting regions of the two proteins.**

**Fig 4: Mammalian Two-Hybrid Analysis of Vif mutants to localise regions involved in interacting with $Pr55^{GAG}$.**

COS-1 cells (~80% confluent) were co-transfected with the Gal4 responsive CAT reporter (pG5CAT), a $Pr55^{GAG}$ fusion construct in the pM Gal4 DNA binding domain vector and various Vif fusion mutants with the designations given in Figure 3 in the pVP16 Gal4 activation domain vector. Negative controls in each case involved co-transfection of only the CAT reporter plasmid and Vif fusion under analysis. The specific plasmids used in each case are indicated under each column of the bar chart using the construct abbreviations given in Figures 3. At three days post transfection the cell monolayers were harvested and the level of reporter CAT expression assayed in cell free extracts using a CAT ELISA assay as described in Materials and Methods.

Panel A: Shows results for the deletion mutants of Vif generated using inverse PCR mutagenesis (IPCRM) as described in Materials and Methods.

Panel B: Shows results for a series of point mutations of the conserved tryptophan at amino acid 21 of Vif. These mutations were generated using site directed mutagenesis.

Panel C: Shows the results obtained on mutagenesis of the highly conserved YLAL residues that form part of the BC-box motif near the carboxy terminus of Vif. These mutations were generated using site directed mutagenesis.

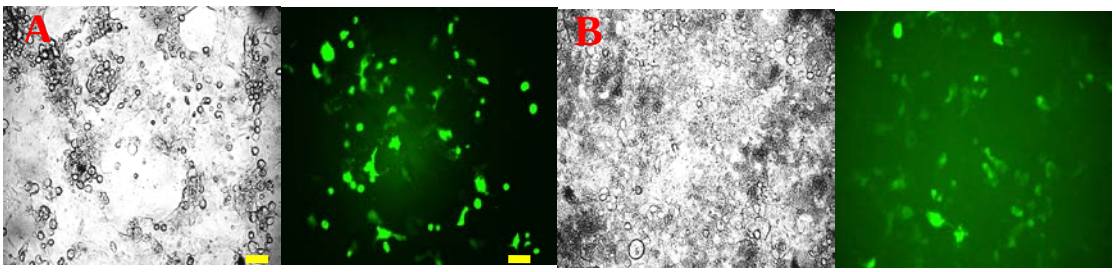
**Fig 5: Mammalian Two-Hybrid Analysis of $Pr55^{GAG}$ mutants to localise regions involved in interacting with Vif.**

COS-1 cells (~80% confluent) were co-transfected with the Gal 4 responsive CAT reporter (pG5CAT), a Vif fusion construct in the pVP16 Gal4 activation domain vector and various $Pr55^{GAG}$ fusion mutants with the designations given in Figure 3 in the pM Gal 4 DNA binding domain vector. Negative controls in each case involved co-transfection of only the CAT reporter plasmid and $Pr55^{GAG}$ fusion under analysis. The specific plasmids used in each case are indicated under each column of the bar chart using the construct abbreviations given in Figure 3. At three days post transfection the cell monolayers were harvested and the level of reporter CAT expression assayed in cell free extracts using a CAT ELISA assay as described in Materials and Methods.

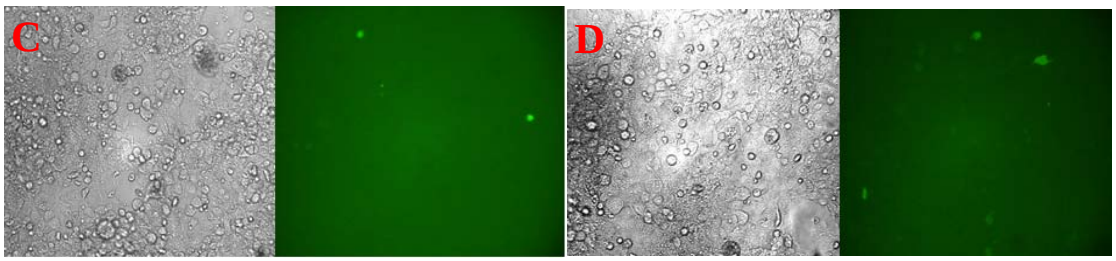
**Fig 6: In–vitro GST Pull down assay of interactions between Vif and $Pr55^{GAG}$.**

This assay was carried out as described in Materials and Methods.

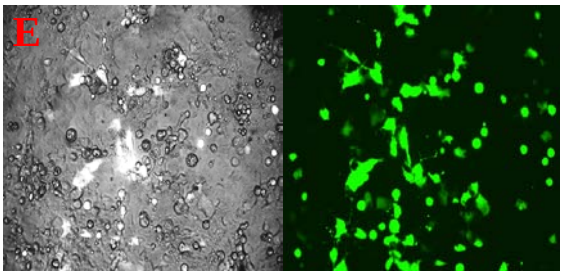
**Figure.1**




AD-Vif+BD-Pr55$^{GAG}$ AD-Pr55$^{GAG}$+BD-Vif




AD-Vif+BD-Protease AD-Pr55$^{GAG}$BD-Protease




+ve control (LargeT+p53)

**Figure.2**

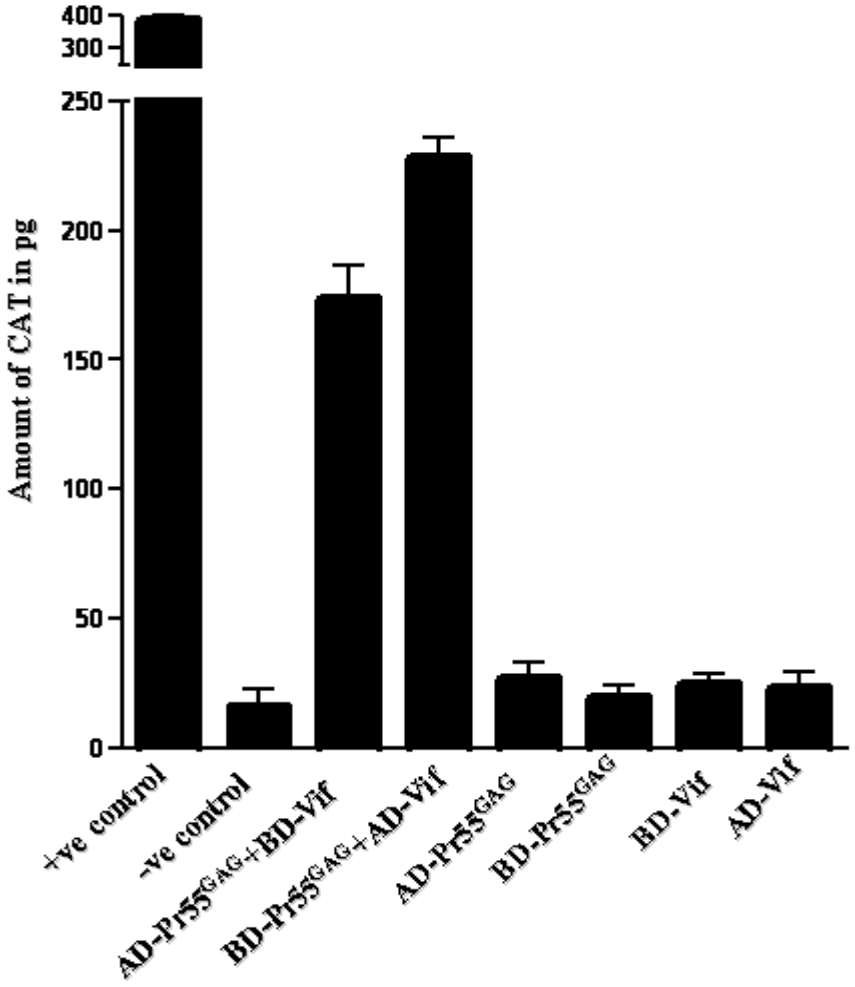
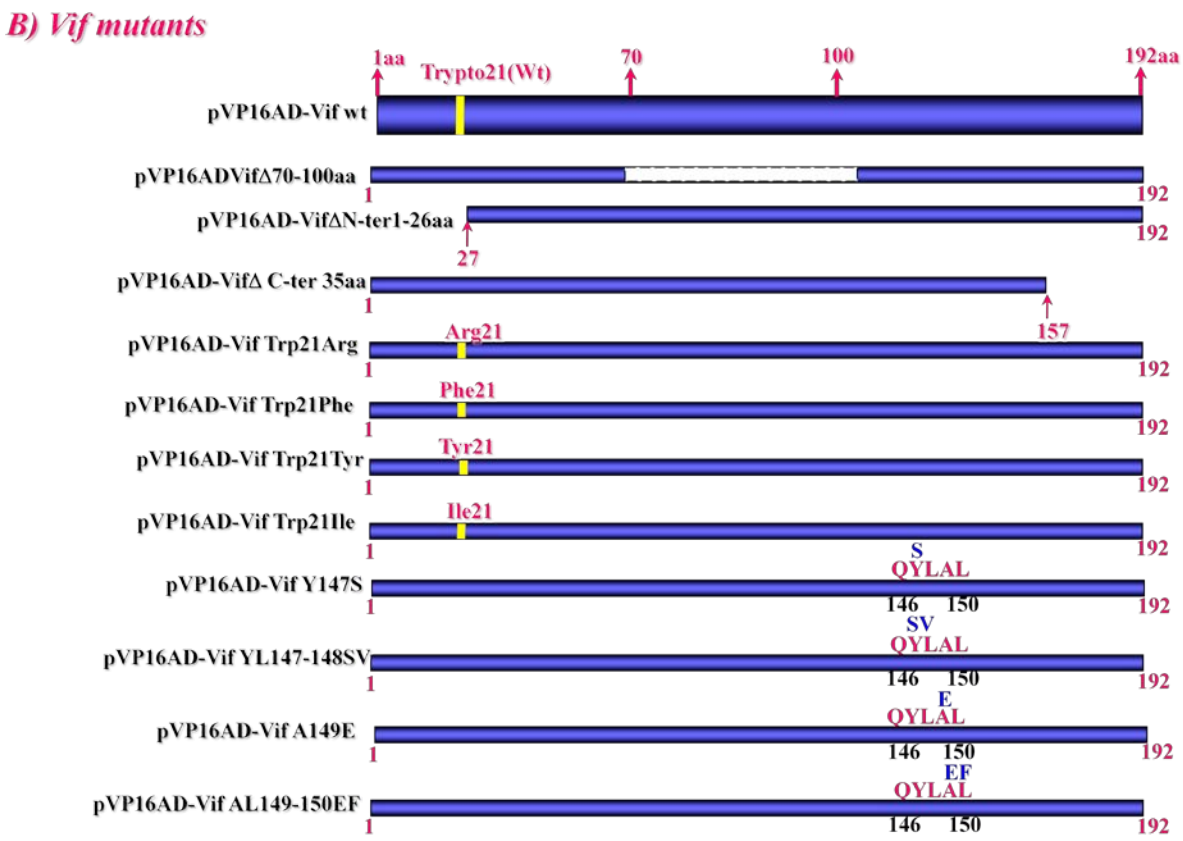

Amount of CAT in pg
400
300
250
200
150
100
50
0
+ve control
-ve control
AD-Pr55GAG+BD-Vif
BD-Pr55GAG+AD-Vif
AD-Pr55GAG
BD-Pr55GAG
BD-Vif
AD-Vif

**Figure.3**


B) Vif mutants
1aa
Trypto21(Wt)
70
100
192aa
pVP16AD-Vif wt
pVP16ADVifΔ70-100aa
1
192
pVP16AD-VifΔN-ter1-26aa
27
192
pVP16AD-VifΔ C-ter 35aa
1
157
pVP16AD-Vif Trp21Arg
Arg21
1
192
pVP16AD-Vif Trp21Phe
Phe21
1
192
pVP16AD-Vif Trp21Tyr
Tyr21
1
192
pVP16AD-Vif Trp21Ile
Ile21
1
192
pVP16AD-Vif Y147S
S
QYLAL
1
146 150
192
pVP16AD-Vif YL147-148SV
SV
QYLAL
1
146 150
192
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1
146 150
192

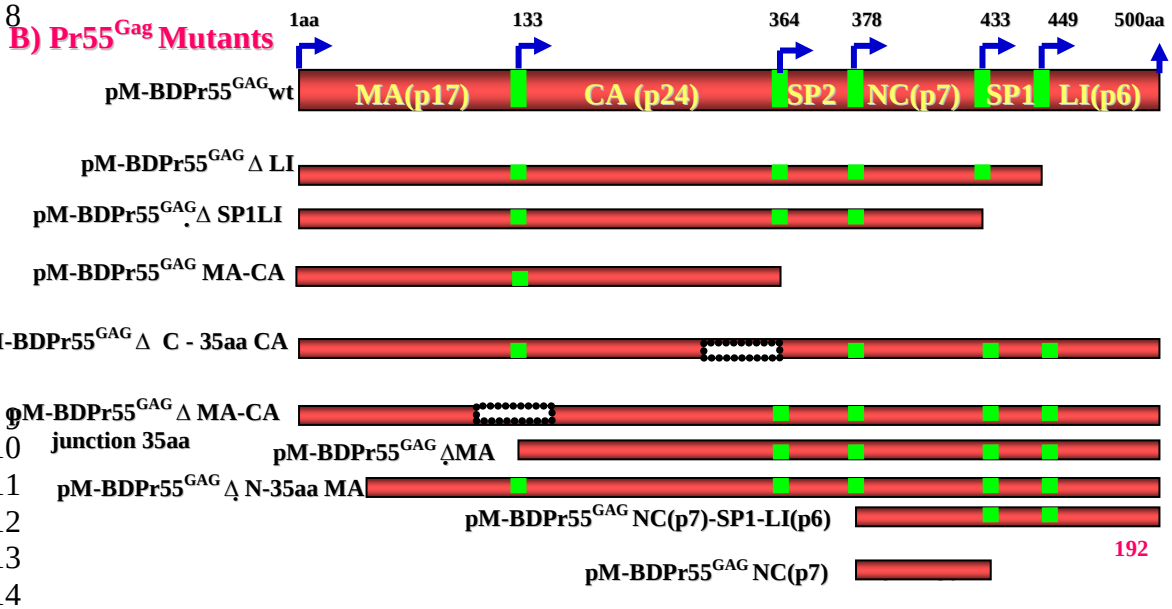
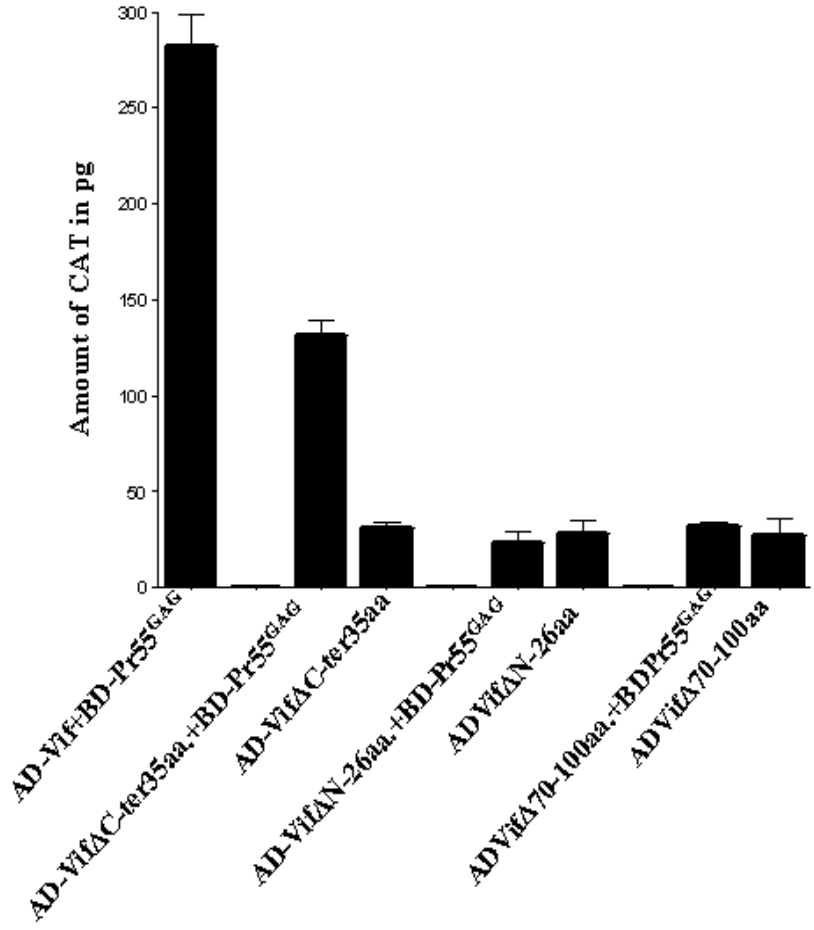
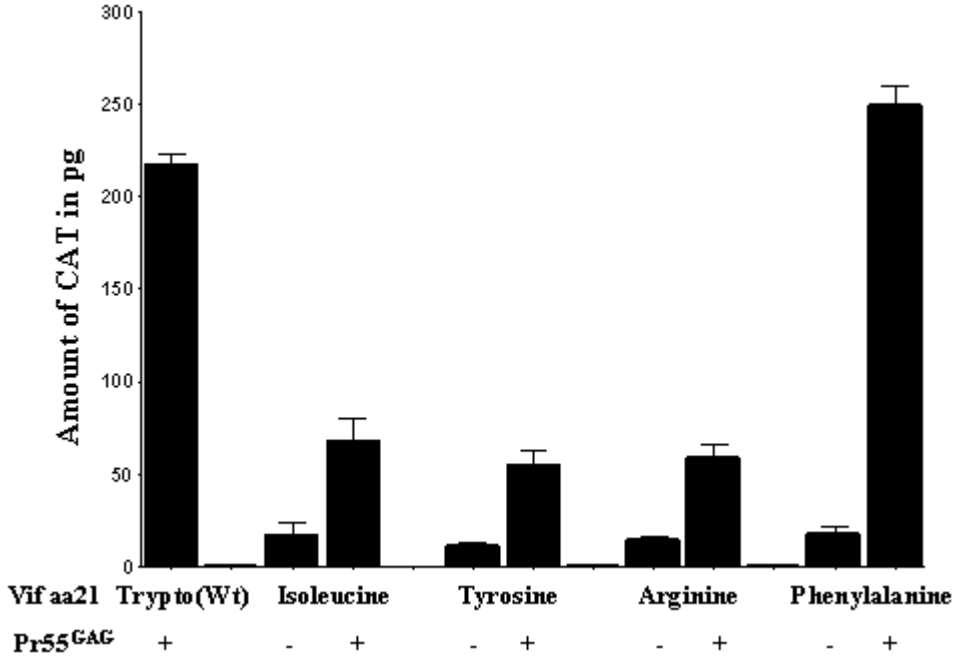

B) Pr55Gag Mutants
1aa
133
364
378
433
449
500aa
pM-BDPr55GAGwt
MA(p17)
CA (p24)
SP2
NC(p7)
SP1
LI(p6)
pM-BDPr55GAG Δ LI
pM-BDPr55GAG Δ SP1LI
pM-BDPr55GAG MA-CA
pM-BDPr55GAG Δ C - 35aa CA
pM-BDPr55GAG Δ MA-CA
junction 35aa
pM-BDPr55GAG ΔMA
pM-BDPr55GAG Δ N-35aa MA
pM-BDPr55GAG NC(p7)-SP1-LI(p6)
192
pM-BDPr55GAG NC(p7)

**Figure.4**

**A.**


Amount of CAT in pg
300
250
200
150
100
50
0
AD-Vif+BD-Pr55GAG
AD-VifΔC-ter35aa.+BD-Pr55GAG
AD-VifΔC-ter35aa
AD-VifΔN-26aa.+BD-Pr55GAG
ADVifΔN-26aa
ADVifΔ70-100aa.+BDPr55GAG
ADVifΔ70-100aa

**B**.


Amount of CAT in pg
300
250
200
150
100
50
0
Vif aa21 Trypto(Wt) Isoleucine Tyrosine Arginine Phenylalanine
Pr55GAG + - + - + - + - +

**C.**

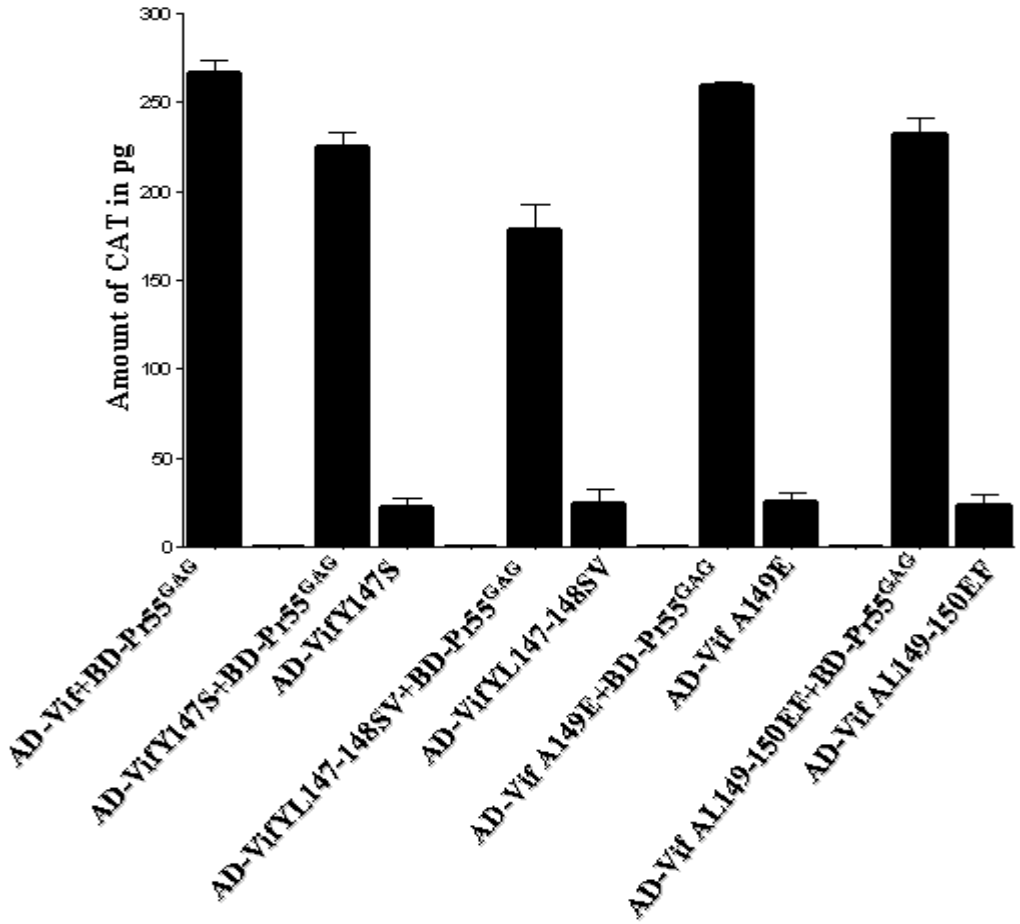

Amount of CAT in pg
300
250
200
150
100
50
0
AD-Vif+BD-Pr55GAG
AD-VifY147S+BD-Pr55GAG
AD-VifY147S
AD-VifYL147-148SV+BD-Pr55GAG
AD-VifYL147-148SV
AD-Vif A149E+BD-Pr55GAG
AD-Vif A149E
AD-Vif AL149-150EF+BD-Pr55GAG
AD-Vif AL149-150EF

**Figure.5**

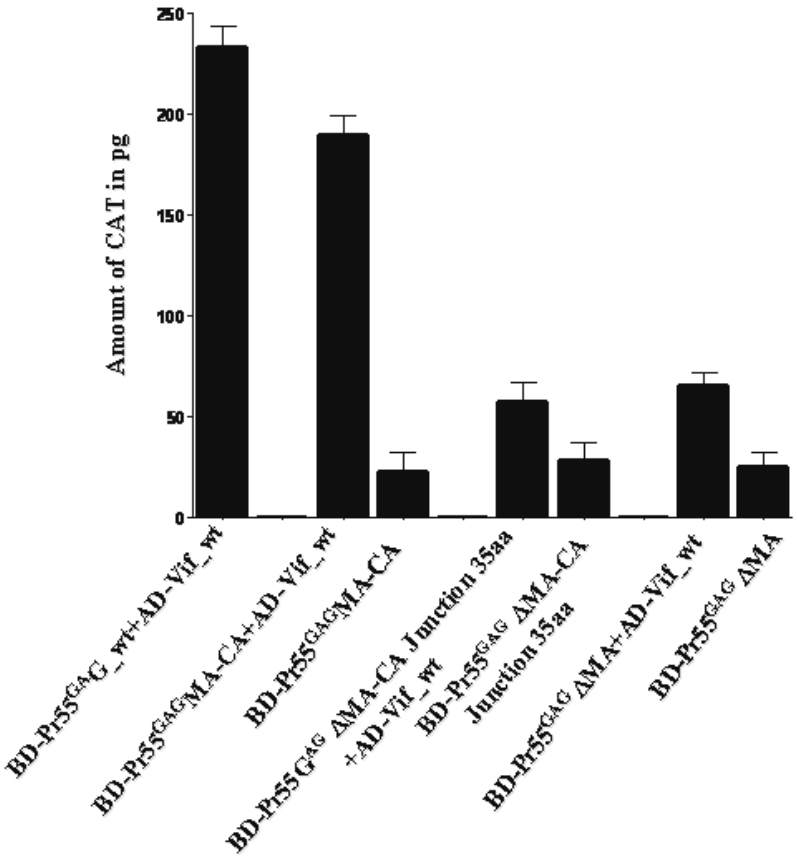
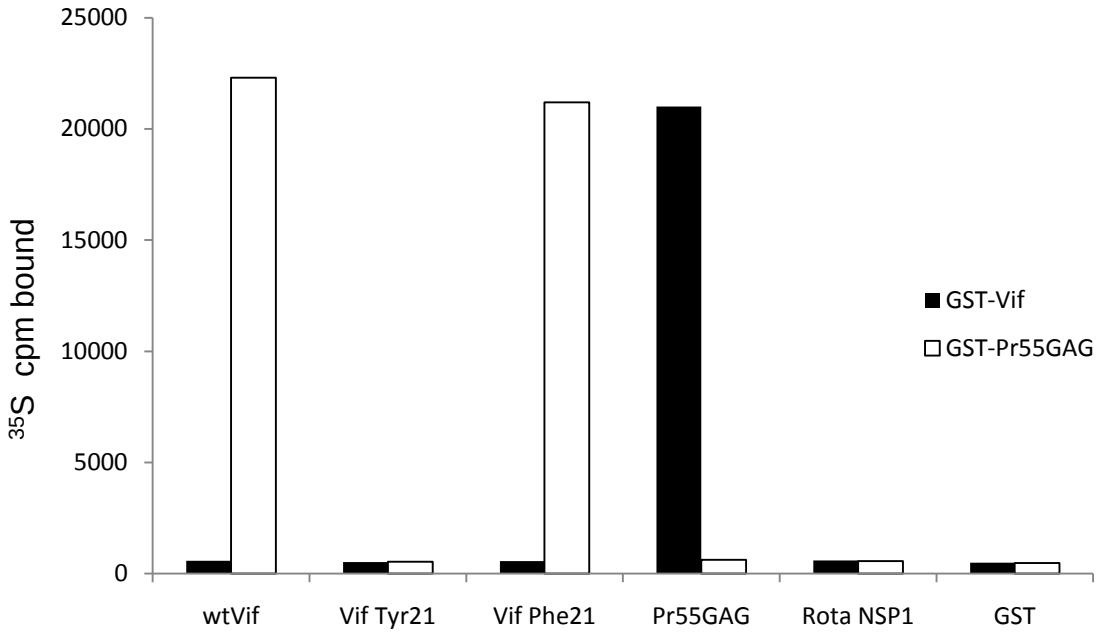

Amount of CAT in pg
250
200
150
100
50
0
BD-Pr55GAG_wt+AD-Vif_wt
BD-Pr55GAGMA-CA+AD-Vif_wt
BD-Pr55GAGMA-CA
BD-Pr55GAG ΔMA-CA Junction 35aa +AD-Vif_wt
BD-Pr55GAG ΔMA-CA Junction 35aa
BD-Pr55GAG ΔMA+AD-Vif_wt
BD-Pr55GAG ΔMA

**Figure 6**


25000
20000
15000
10000
5000
0
35S cpm bound
GST-Vif
GST-Pr55GAG
wtVif
Vif Tyr21
Vif Phe21
Pr55GAG
Rota NSP1
GST

1
2
3
4
5

1
2

| Pr55$^{GAG}$-DB and Vif-AD | GFP | CAT |
|---|---|---|
| Pr55$^{GAG}$ (wt) + Vif (wt) | ++++ | ++++ |
| pM-BDPr55$^{GAG}$MA-CA+Vif (wt) | +++ | +++ |
| pM-BDPr55$^{GAG}$ΔMA-CA junction 35aa +Vif (wt) | - | - |
| pM-BDPr55$^{GAG}$ΔMA +Vif (wt) | - | - |
| pM-BDPr55$^{GAG}$ΔSP1LI +Vif (wt) | ++++ | ND |
| pM-BDPr55$^{GAG}$ΔC-35aaCA +Vif (wt) | ++++ | ND |
| pM-BDPr55$^{GAG}$ΔN-35aaMA +Vif (wt) | ++++ | ND |
| pM-BDPr55$^{GAG}$NC(p7)-SP1-LI(p6)+Vif (wt) | - | ND |
| pM-BDPr55$^{GAG}$ΔLI +Vif (wt) | ++++ | ND |
| pM-BDPr55$^{GAG}$NC(p7) +Vif (wt) | - | ND |

**Table 1 Summary of Mammalian Two-Hybrid Results Pr55$^{GAG}$ Mutants.**

A series of Pr55$^{GAG}$ deletion mutants in the Gal4 DNA Binding Domain vector were constructed by inverse PCR mutagenesis [30]. Mutants were tested for their interaction with wild type (wt) Vif expressed as fusion with the VP16 Gal4 Activation Domain using GFP and CAT as a reporter. Comparison of the expression of GFP or CAT reporter was done in relation to that given by a Wild type Pr55$^{GAG}$ and wild type Vif interaction which was considered to be 100% (++++). Where ++++ = 100%; +++ = <75%; ++ = <50%; + = <25%; - = 0% and ND = not done.

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