Transactions on Materials, Biotechnology and Life Sciences
ISSN: 2960-1932, eISSN: 2960-2319 | Volume 7
MISBP 2024

Genome editing of PSIP1 gene encoding LEDGF/p75 protects TZM_bl GFP cells from HIV-1 infection

Kerong Pang*

School of Medicine, Imperial College London, London, the United Kingdom

*Corresponding author: kerongandreapang@outlook.com

Abstract. Gene therapy is a good way to cure HIV permanently. Human immunodeficiency virus (HIV-1) infected cells depend on host-cell factors. LEDGF/p75 is a co-factor encoded by the *PSIP1* gene and it interacts with HIV integrase to help the virus bind to host cells genome. If the *PSIP1* gene is knocked out in the TZM-bI GFP cell line using CRISPR/Cas technology, the amount of HIV DNA integrated into the cell will be reduced due to the inability of HIV integrase to efficiently bind and integrate into the transcriptionally active regions of the host cell chromatin. The change in infectivity was observed by fluorescence microscopy and flow cytometry. The results show that KO *PSIP1* may inhibit HIV infectivity. More experiments are needed to prove the reliability of this result..

Keywords: PSIP1, LEDGF, HIV-1, CRISPR/Cas, TZM_bl GFP cells, Infectivity assay, Western blot, Flow cytometry, Immunofluorescence.

1. Introduction

Acquired immunodeficiency syndrome (AIDS) is an acquired immune disease. It is caused by human immunodeficiency virus (HIV). So far, many studies have identified several targets in therapy. For example, combined antiretroviral therapy (cART) prevents the virus from replicating in the body.[1] This controls the progression of the disease by reducing the viral load. With cART, it takes more than 70 years of continuous treatment for HIV to be eradicated.[2] Therefore, gene-editing therapy has been discovered. For example, the C-C motif chemokine receptor type 5 (CCR5) has been successfully edited as a target, thus inhibiting the invasion of HIV-1 which demonstrates the possibilities of gene-editing therapies.

Lens epithelium-derived growth factor p75 (LEDGF/p75) is a transcriptional coactivator, consisting of gene PC4 and SFRS1-interacting protein 1 (*PSIP1*).[3] It was found that LEDGF/p75 interacts with HIV integrase (IN) and can assist in the binding of the virus to host cells genome. Moreover, LEDGF/p75 stimulates catalytic activity of IN and protects it from proteolytic degradation.[4] This study aims to investigate the effect of knocking out (KO) *PSIP1* on TZM_bl GFP cells. If the *PSIP1* gene is knocked out in the TZM-bI GFP cell line using CRISPR/Cas technology, then integration of HIV DNA into the cell will decrease due to HIV integrase being unable to efficiently bind and integrate into transcriptionally active regions of host cell chromatin. Here, western blot was used to make sure *PSIP1* successfully KO, but results showed potential KO for *PSIP1*. The effect of successful KO of *PSIP1* on HIV infection was detected by fluorescence microscopy and virus entry was measured by flow cytometry. However, the infectivity assay can prove that *PSIP1* KO reduced HIV infection. But there was shown a clear trend to prove that KO *PSIP1* can reduce p24 expression. There is no research that directly demonstrate the mechanism by which KO of *PSIP1* can reduce HIV infection. This may provide a new avenue for HIV-1 treatment.

2. Materials and Methods

2.1. Generation of stable knockout PSIP1 cell lines by CRISPR/Cas

Guide RNA (gRNA) were generated by CRISPR and ligated into CRISPR/Cas9 (pSF-CMV-Cas9, 279 bp) plasmid. The pSF-CMV-Cas9 (0.5 μg/μl; Addgene, diluted to 200 μg/mL), BsaI (20,000

U/ml; New England Biolabs; NEB; diluted to 1000 U/mL) and CutSmart Buffer (10X, NEB) were used to plasmid linearisation. The target and non-targeting gRNA sequences (NTC) were used in CRISPR to create knockout *PISP1* and NTC cell clones (Table 1). Then, 10X TAE buffer (Invitrogen; diluted to 1X) and agarose (Appleton Woods) with GelGreen (Cambridge Bioscience) were used to form 2% agarose gel. Forward and reverse g RNA oligo (100 μm) were annealed, after that, plasmid was ligated by T4 DNA Ligase Reaction Buffer (10x, NEB; diluted to 1X) and T4 DNA Ligase (400,000 U/ml, NEB; diluted to 20000 U/mL), and transferred to bacteria. Then, for colony PCR, U6 Forward primer (10 μM, Sigma Aldrich, 5'-3') was added, and that plasmid was expanded to bacterial cultures and purification and transfected to mammalian cells which need to be diluted by Opti-MEM (Thermo Fisher Scientific; TFS) at room temperature and Viafect (Promega) was added, then incubated for 48h to check GFP signals. All steps were verified by Sanger Sequencing (performed by Azenta Life Sciences, UK).

Table 1: The sequences for *PSIP1* and non-targeting gRNA used in CRISPR to create knockout PSIP1 and NTC cell clones.

Target	***PSIP1***
gRNA 1	AAACACCGCAATGGATTCTCGACTTCAAGTTTTAGAG
gRNA 2	AAACACCGGTAGCTGCGCTGCTCCCGCGGTTTTAGAG
gRNA 3	AAACACCGAAGAGCCGGATAAAAAAGAGGTTTTAGAG
gRNA 4	AAACACCGAATCGCGAGTCATGTTTCGGGTTTTAGAG
Non-Targetting gRNA	5' GTATTACTGATATTGGTGGG 3' (manufacturer: Sigma Aldrich)

2.2. Western blot

KO cells and NTC cells were centrifuged and washed 3 times with 1X PBS (TFS) and added lysis buffer and protease (10x, TFS) and phosphatase (10x, TFS) inhibitors as 10:1:1 ratio. Then, the proteins were quantified as normal protocols. 15 μg protein for each well was used for running 4-12% Bis-Tris gel (Mini Protein Gel, 15-well, Invitrogen). Then, proteins were transferred to a nitrocellulose membrane (BioRad). The membrane was stained using ponceau S solution (Sigma-Aldrich) and blocked for 30 mins by 5% mike-powder in 1X Tris-buffered saline-Tween (TBS-T) which was made by 10X TBS (Sigma-Aldrich) and Tween-20 (Sigma-Aldrich). According to the protein ladder, the membrane was cut into two parts for primary antibody incubation. *PSIP1* monoclonal antibody (TFS, #MA5-15795) and Histone (H3) antibody (CST, #9715S) were used at 1:1000 ratio and incubated for 2 hours at room temperature, respectively. After washing 3 times in 1X TBS (Sigma-Aldrich), the membrane was incubated with a secondary antibody which is Polyclonal Goat Anti-Rabbit Immunoglobulins/HRP (Dako/Agilent, #P044801-2) at 1:2000 ratio for 1h at room temperature. After 3 times washing using 1X TBS (Sigma-Aldrich), protein signals were detected by Super Signal ECL (TFS) for 5 mins and imaged by iBright Imaging Systems (TFS). The membrane was cropped and adjust by ImageJ.

2.3. Infectivity assay

Firstly, cells in 24-well plate were seeded as seeding density for 2 days culture and infected by virus-like particles (VLP; made by LP2 Teaching Team). Then, the cells were washed with 37°C 1X PBS (TFS) 3 times. 4% Paraformaldehyde (TFS) was added to each well and incubated for 20 mins by GTA. After 3 times washing by 1X PBS (TFS), DAPI (1 μg/ml, TFS) was added for staining and incubated for 10 minutes covered with foil. The cells were washed with 1X PBS (TFS) and added back. Fluorescence microscope Axiovert 5 (Zeiss) was used to observe the cells, and pictures were adjusted and counted by Fiji ImageJ. The formula for GFP percentage is %GFP = number of GFP/number of DAPI.

2.4. Flow cytometry

The cells in 6-well plate were seeded as seeding density for 2 days culture and infected by VLP (made by LP2 Teaching Team). Then 1X PBS (TFS) was used to wash once. TrypLE Express Enzyme (1X, TFS) were added and incubated for 5 mins to dissociate cells, then added 10% Fetal bovine serum (FBS, TFS) and Penicillin-Streptomycin (TFS) to neutralise it. The cells were centrifuged at 1500 rpm for 5 mins. After 2 times washing by 1X PBS (TFS), the cells were added 10X IC Fixation Buffer (TFS) and incubated for 20 mins by GTA. Then, permeabilization buffer (10X, TFS; diluted to 1X) was added to each tube. After centrifuging, the cells were resuspended and AlexaFluor647-conjugated p24 antibody (Santa Cruz, #sc-69728 AF647) was added to full stain samples in 1:100 and incubated at 4^{o}C for 1 hour. The cells were washed with 1X permeabilization buffer (TFS) 2 times and resuspended the cell pellet in ice-cold FACS staining buffer (TFS). Then it was transferred to FACS tubes (SLS) and proceeded to data acquisition by flow cytometer (BD Accuri C6 Plus) and analysis by BD Accuri C6 software.

3. Results

3.1. Identification of PSIP1 KO in TZM_bl GFP cell line

Confirmation of successful KO with CRISPR/Cas9 *PSIP1* in TZM_bl GFP cell line is important for the next experiments. Western blot was used to detect two single clones of monoclonal cells (CL1 and CL2) after *PSIP1* KO and the NTC TZM_bl GFP cell line. Histone 3 (H3) was used as a loading control to determine protein loading variation (Figure 1). As for LEDGF/p75, after normalisation with H3, there were no bands here. This means that the LEDGF/p75 expression is at undetectable levels. For CL1b, H3 loading density was different than others. Overall, the result is invalid, which means that *PSIP1* is a potential KO for these two TZM_bl GFP cell lines.

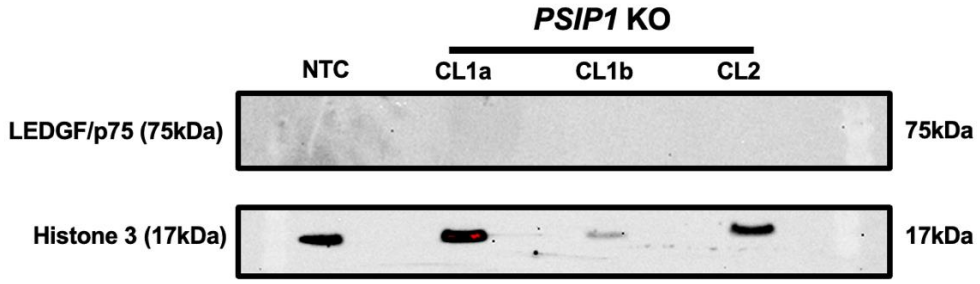

Figure 1: The identification *of* the tzm_bl GFP cell line KO *PSIP1* by using Western blot to analysis of LEDGF/p75 protein in 2 different *PSIP1* KO clones. n=1

3.2. PSIP1 KO inhibit TZM_bl cell infectivity shown by GFP and p24 levels

To determine that *PSIP1* KO was effective in reducing the infectivity of VLP on TZM_bl GFP cell lines, fluorescence microscopy was used to observe the percentage of GFP-expressing. The GFP expression of KO and NTC cells of VLP+ and KO and NTC cells of VLP- were compared, respectively (Figure 2a and b). It can be found that NTC/+VLP had the highest GFP percentage, which is about 2-fold higher than that of CL1/+VLP (Figure 2c). And all VLP infected cells showed more GFP signals compared with uninfected VLP. This suggests that *PSIP1* KO can inhibit HIV-1 infection to a certain extent. However, since GFP signals were also observed in cells with uninfected VLP, this proves that the experimental results are not accurate.

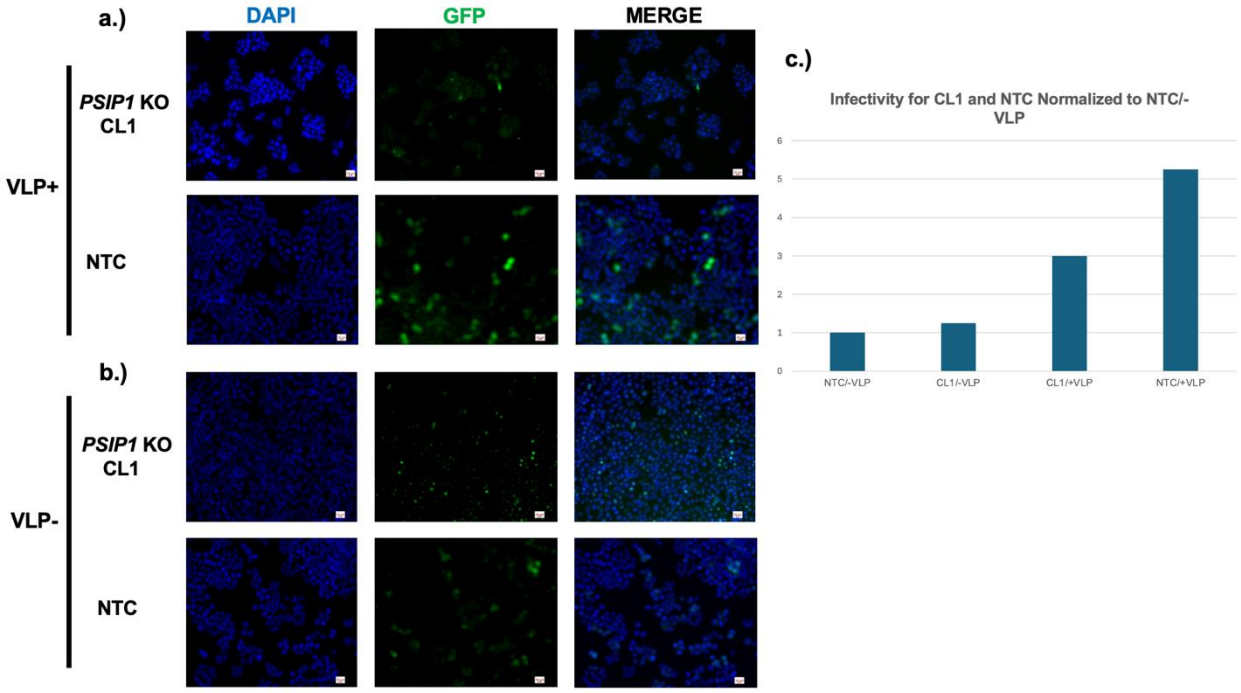

Figure 2: The infectivity for VLP *infected* tzm_bl GFP cell line KO *PSIP1* and NTC cell clones by using fluorescence microscopy. Scale Bar: 10 µm. n=1

a.) Infectivity of *PSIP1* KO and NTC cell clones after infection by VLP.

b.) Infectivity of *PSIP1* KO and NTC cell clones not infected by VLP.

c.) Infectivity of *PSIP1* KO and NTC cell clones normalised to NTC/-VLP infectivity.

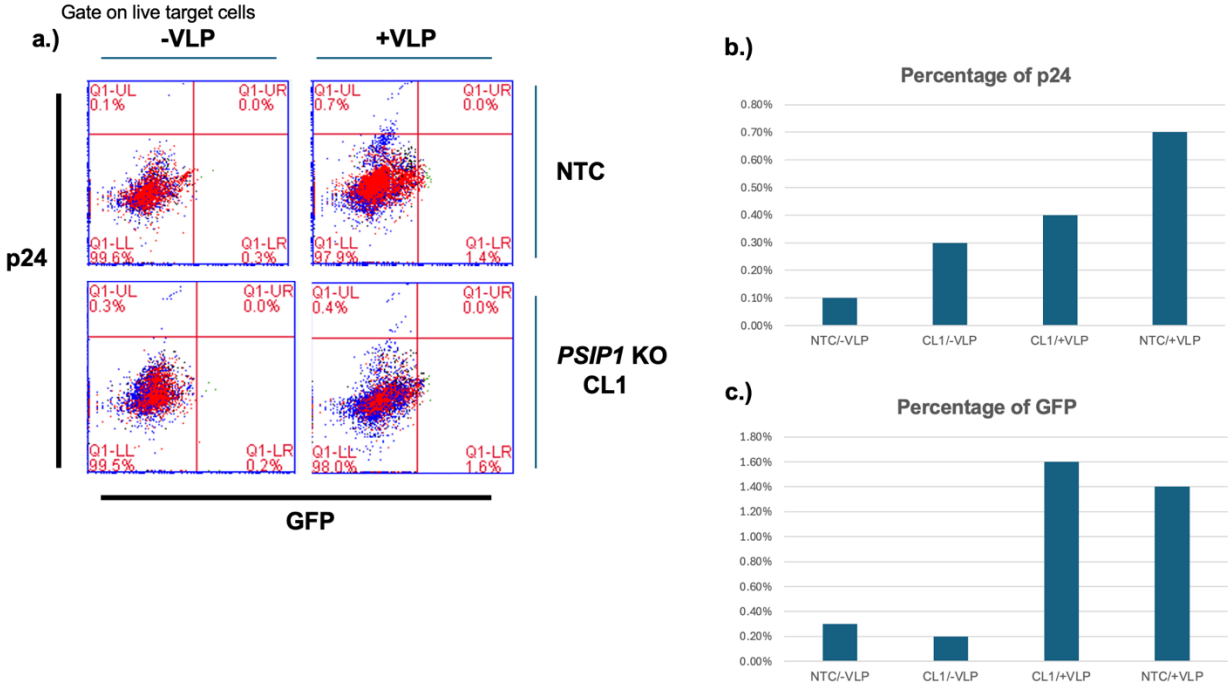

Figure 3: Effect of VLP infection of *tzm*_bl GFP cell line on p24 and GFP in KO *PSIP1* and NTC cell clones by flow cytometer. Five different gates were used, they were: FSC/SSC gated the live population of cells. FSC-A/FSC-H was used to remove cell doublets. GFP/SSC gated the GFP expression cells. p24/SSC gated the p24 expression cells. GFP/p24 was used to show the cells express both GFP and p24. But only one gate, GFP/p24, is shown here. n=1

a.) Representative flow cytometry plots of uninfected and infected *PSIP1* KO and NTC cells stained with GFP and AlexaFluor647-conjugated HIV-1 p24 Antibody.

b.) Percentage of p24 infected or uninfected *PSIP1* KO and NTC cell clones.

c.) Percentage of GFP infected or uninfected *PSIP1* KO and NTC cell clones.

To further confirm which step of the HIV infection cycle *PSIP1* plays a role flow cytometry was used to test the p24 and GFP expressing percentages of TZM_bl GFP cell clones at viral entry by detecting the effects of VLP infection on NTC and *PSIP1* KO (Figure 3). Figure 3a showed a comparison of p24 and GFP expression under four different conditions in a flow cytometry plot. The percentage in each quadrant indicates the proportion of cells in each population. It can be found that the p24 expression of NTC/+VLP was the highest and 1.5-fold higher than that of CL1/+VLP (Figure 3b). This suggests that KO *PSIP1* can prevent HIV infection at entry. However, for GFP expression, CL1/+VLP had the highest percentage (Figure 3c). And no group of cells in Figure 3a showed GFP+ cells that express p24 (0%). This suggests that the results of this experiment are not reliable enough.

4. Discussion and conclusion

Although antiretroviral drugs for controlling HIV replication have been effective, turning HIV into a chronic disease has reduced mortality. It is still not possible to eradicate HIV. Protecting uninfected HIV target cells through site-specific gene targeting or gene therapy reduces the infection of cells by HIV-1.[5] Through previous studies, it has been shown that LEDGF/p75 can bind to HIV IN proteins, leading to a reduction in the integration of transcription units.[6] Therefore, KO *PSIP1* can disrupt HIV-1 replication. CRISPR/Cas9 was used to edit the gRNA, and the *PSIP1* gene comprising LEDGF/p75 was knocked out of the gRNA to generate a stable *PSIP1* KO cell line.

According to the results section of this thesis, it can be found that the western blot results did not provide good evidence that *PSIP1* was successfully KO, and infectivity assay and flow cytometry did not produce reliable results to prove that KO *PSIP1* could inhibit the infection of VLP. However, according to investigation, there is evidence from other teams that *PSIP1* can be successfully KO, and that it can inhibit HIV infection by immunofluorescence. As for our results, the fact that NTC does not show bands may be due to a problem with the protein sample produced. The band density of H3 for CL1b is not same as others. The loading amount of the protein sample needs to be recalculated. In the future, more biological replication is needed, and the limitation mentioned above should be optimised. For the infectivity assay, comparing CL1/+VLP and NTC/+VLP showed a good trend indicating that *PSIP1* KO can inhibit infectivity. However, all uninfected cells also showed GFP expression. This may be due to contamination of cell cultures with GFP-expressing vectors or cells. In addition, autofluorescence from the cells themselves may be mistaken for GFP.[7] Therefore, more replication is needed to further demonstrate the reduced infectivity. Due to LEDGF/p75 can repair double-strand break (DSB)[8], and HIV infection will also increase DBS. Immunofluorescence determines the infectivity of the integration part by measuring DSB. Therefore, it is impossible to determine whether the increase in DSB is due to LEDGF/p75 KO or HIV infection. But for flow cytometry, since p24 is part of the capsid of VLPs, it should be detected when VLPs enter.[9] Although p24 demonstrated *PSIP1* KO, p24 decrease, GFP expression could not prove that *PSIP1* KO decreased HIV infection. The KO LEDGF/p75 may affect other mechanisms in the entry so that p24 decreases but GFP is slightly higher. Many cells showed a GFP-/p24- quadrant, and these 2 signals percentage were both small. This may indicate that cell viability is too low. New KO *PSIP1* cell clones and more replications are needed to determine reliability of the results. Immunofluorescence can also be performed, and the results compared to further determine whether KO LEDGF/p75 mainly affects virus entry or integration.

Gene therapy is a promising treatment for HIV, and CRISPR/Cas is a useful gene editing tool. KO LEDGF/p75 has been preliminarily shown to reduce HIV infection. In this study, more experimental replications is needed in the future and many limitations need to be optimised. Thus, more reliable results will be produced to show that if the *PSIP1* gene is KO in the TZM_bl GFP cell line, the HIV infection will be suppressed.

Acknowledgements

Thanks to all fellow students and our team members for their help and contribution. Thanks to Team 23 and Team 7 for providing cell clones. Thanks for the HIV1 Virus-like particles were prepared in-house by the LP2 Teaching Team using plasmids (pVSV-G, p8.91 and pCSGW-GFP) provided by the Retrovirus-Host Interactions Lab- The Crick Institute-London, United Kingdom. Also, thanks to Teaching Fellow for all helps, especially our supervisor – Annachiara Rosa. Thanks to all GTA for helping with fixing cells, tissue culture and fluorescence microscopy, and all technical staff for helping with using technique. Thanks, Azenta Life Sciences UK for doing Sanger Sequencing, and the LMS/NIHR Imperial Biomedical Research Centre Flow Cytometry Facility (Hammersmith Hospital, London for sorting transfected cells. Thanks to the Benchling website, which helped organise the data.[10]

References

[1] Liu, Zhepeng et al. “Genome editing of the HIV co-receptors CCR5 and CXCR4 by CRISPR-Cas9 protects CD4+ T cells from HIV-1 infection.” Cell & bioscience vol. 7 47. 9 Sep. 2017, doi:10.1186/s13578-017-0174-2

[2] Qi J, Ding C, Jiang X, Gao Y. Advances in developing CAR T-Cell therapy for HIV cure. Frontiers in Immunology [Internet]. 2020 Mar 10;11. Available from: https://doi.org/10.3389/fimmu.2020.00361

[3] LEDGF: A Leukemia-Specific Target | Blood | American Society of Hematology,ashpublications.org/blood/article/131/1/4/107677/LEDGF-a-leukemia-specific-target. Accessed 21 June 2024.

[4] Blokken J, De Rijck J, Christ F, Debyser Z. Protein–protein and protein–chromatin interactions of LEDGF/p75 as novel drug targets. Drug Discovery Today Technologies [Internet]. 2017 Jun 1;24:25–31. Available from: https://www.sciencedirect.com/science/article/pii/S1740674916300476?via=ihub

[5] Cillo AR, Mellors JW. Which therapeutic strategy will achieve a cure for HIV-1? Current Opinion in Virology [Internet]. 2016 Jun 1;18:14–9. Available from: https://www.sciencedirect.com/science/article/pii/S1879625716300050?via%3Dihub

[6] Ciuffi A, Diamond TL, Hwang Y, Marshall HM, Bushman FD. Modulating target site selection during human immunodeficiency virus DNA IntegrationIn vitrowith an engineered tethering factor. Human Gene Therapy [Internet]. 2006 Sep 1;17(9):960–7. Available from: https://doi.org/10.1089/hum.2006.17.960

[7] Kain SR, Kitts P. Expression and detection of Green Fluorescent Protein (GFP). In: Humana Press eBooks [Internet]. 2003. p. 305–24. Available from: https://doi.org/10.1385/0-89603-481-x:305

[8] Liedtke V, Schröder C, Roggenbuck D, Weiss R, Stohwasser R, Schierack P, et al. LEDGF/p75 Is Required for an Efficient DNA Damage Response. International Journal of Molecular Sciences [Internet]. 2021 May 30 [cited 2024 Mar 21];22(11):5866. Available from: https://pubmed.ncbi.nlm.nih.gov/34070855/

[9] Anderson AM, Tyor WR, Mulligan MJ, Waldrop-Valverde D, Lennox JL, Letendre SL. Measurement of Human Immunodeficiency Virus p24 Antigen in Human Cerebrospinal Fluid With Digital Enzyme-Linked Immunosorbent Assay and Association With Decreased Neuropsychological Performance. Clinical Infectious Diseases [Internet]. 2018 Jan 29;67(1):137–40. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6005050/#:~:text=The%20HIV%20p24%20antigen%20is

[10] Benchling. Lab Pod 2. Biology Software, [2024, 06, 21]. Available from: https://benchling.com