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In vitro PCR-based screening confirms the absence of HIV genome in healthy volunteers that recovered from COVID-19

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Human immunodeficiency virus (HIV) and coronavirus disease-2019 (COVID-19) were pandemics over the past few decades. A new hypothesis of man-made virus focused on genetically engineered viral genomes using the HIV genome as a backbone emerged during the COVID-19 pandemic. In this study we hypothesized that if the corona viral genome was engineered based on the HIV backbone then it is possible to detect the backbone from healthy volunteers that fully recovered from the COVID-19 infection. HIV being a retrovirus, the viral genome usually is incorporated into the host genome. Hence, a sensitive technique such as polymerase chain reaction (PCR) can detect the presence of HIV backbone in the host blood cell genomic DNA. Healthy volunteer blood samples were used for DNA extraction followed by the PCR-amplification of HIV genes (if any). Our results indicate that none of the samples contained HIV backbone thus confirming that the corona viral genome may have not been engineered using the HIV backbone. However, a large dataset of such volunteer samples should be tested in the future in order to further confirm the current preliminary data.

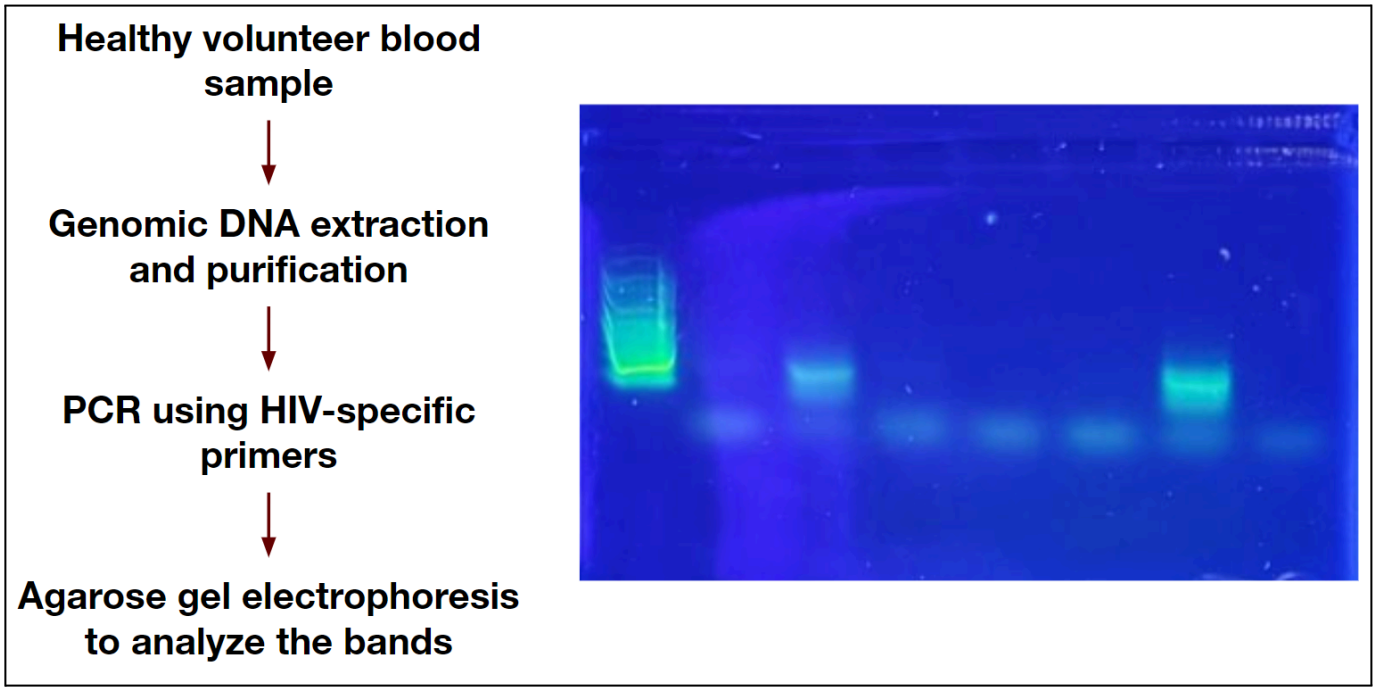

Figure 1. Overall process of PCR-screening to identify the HIV genes.

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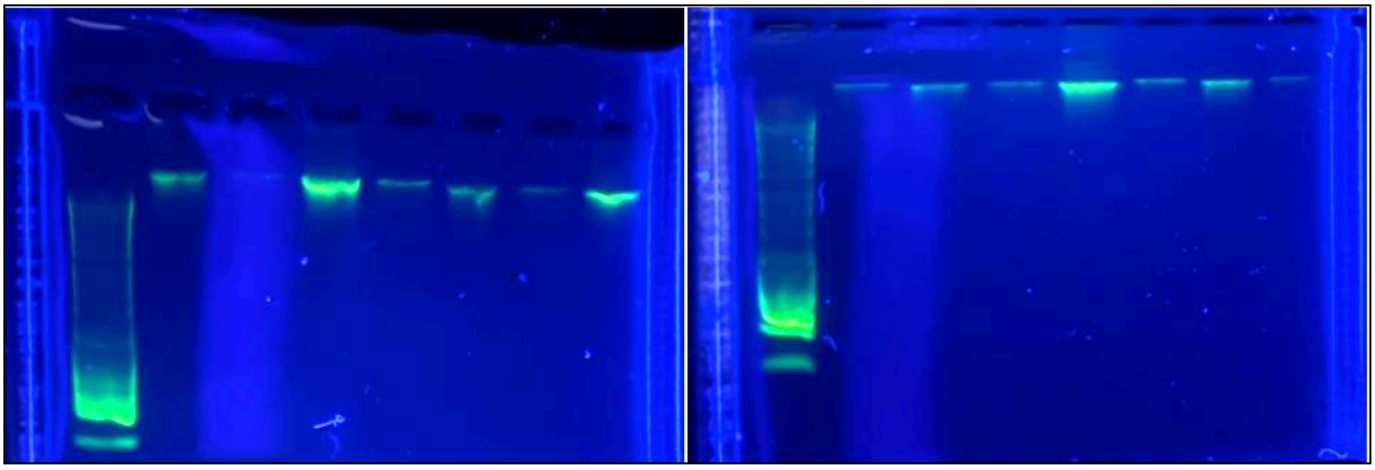

Figure 2. Agarose gel electrophoresis results showing the high molecular weight genomic DNA bands. The first lane in both left and right panels show the standard 1kb DNA ladder.

The coronavirus disease-2019 (COVID-19) caused a huge global pandemic in recent years claiming millions of lives across the globe [1]. The viral spike protein trimers play a critical role in docking to the host cell angiotensin converting enzyme-2 (ACE2) receptors [2, 3]. Multiple approaches were used to design effective vaccines to prevent COVID-19 [4-6]. In addition to the mutation-induced structural changes in the receptor binding domain (RBD) of the viral spike protein, the liquid-liquid phase separation characteristics of RBD are challenging the existing vaccine strategies [7-9]. During the COVID-19 pandemic many theories of the viral emergence, for instance, man-made coronavirus using HIV backbone, appeared in the social media that raised many concerns among the public [10].

Acquired immunodeficiency syndrome (AIDS) caused by the human immunodeficiency virus (HIV) was a pandemic in the 80s leading to huge morbidities [11]. Rapid and error-prone replication of HIV leads to the accumulation of mutations in the viral proteins causing multidrug-resistance [12]. Antiretroviral therapy has significantly helped in controlling the spread of AIDS and/or HIV infections if the regimen was properly followed by the patients [13]. HIV primarily contains two main genes, Gag & Pol that are dominant in the entire viral genome [14, 15].

In this study we hypothesized that if the coronaviral genome was engineered based on the HIV backbone then it is possible to detect the HIV backbone from healthy volunteers that recovered from the COVID-19 infection. HIV being a retrovirus, the viral genome usually is incorporated into the host genome [16]. Hence, a sensitive technique such as PCR can detect the presence of HIV backbone in the host blood cell genomic DNA (Figure 1). Healthy volunteer blood samples were used for DNA extraction followed by the PCR-amplification of HIV genes (if any).

Materials & Methods:

Isolation, purification and qualitative analysis of blood-derived genomic DNA: Blood genomic DNA was extracted and purified using the HiPurA Blood Genomic DNA Miniprep Purification Kit purchased from HiMedia (Cat. No. MB504). DNA samples were run on a 1% agarose gel for qualitative analysis. The standard 1kb DNA ladder was used in the gel to compare the size of the high molecular weight genomic DNA.

PCR-screening and qualitative analysis: PCR was performed using the purified genomic DNA samples. The EmeraldAmp GT PCR Master Mix from TaKaRa was used for PCR amplification. The annealing temperature was systematically scanned between 50 ℃-60 ℃, one degree at a time. Four genomic DNA templates were used and each one was scanned with 11 different annealing temperatures. Thus, a total of 44 reactions were performed. The PCR products were qualitatively analyzed on 1% agarose gel for qualitative analysis. The standard 1kb DNA ladder was used in the gel to compare the size of the high molecular weight genomic DNA.

Sanger sequencing and Bioinformatics: The PCR products were outsourced to Barcode Biosciences, Bengaluru, for Sanger sequencing analysis. Raw chromatograms were analyzed and sequence was finalized by base calling. Thus obtained sequences were then used to search in the NCBI BLASTn in order to identify the target genes. BLASTn search was performed using an “E” value of 0.00 as a cutoff in order to increase the stringency of homology between the query and target sequences in the NCBI database. The top 100 or <100 results were sorted based on the “E” value of each match.

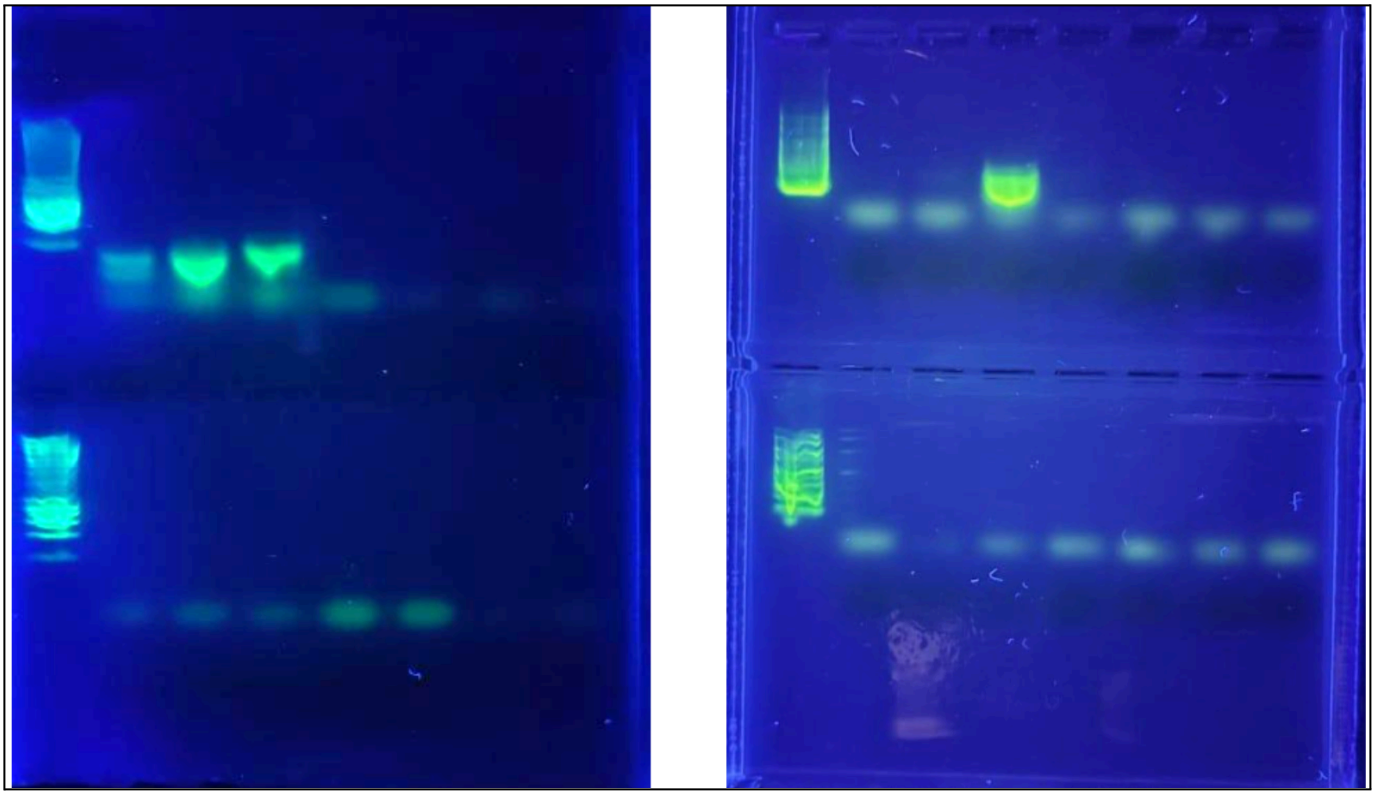

Figure 3. Agarose gel electrophoresis analysis of PCR products.

Results and Discussion:

Qualitative analysis revealed good quality of genomic DNA: The quality of the blood-derived genomic DNA samples was found to be good. As shown in Figure 2, none of the DNA samples exhibited any traces of degradation. However, the quantity of the DNA varied from sample to sample. Presence of clean single bands above the 1kb DNA ladder is indicative of intact and high molecular weight of the evaluated DNA samples in this study. Lack of degradation avoids non-specific PCR-amplifications. These genomic DNA samples were further used as templates for the PCR-screening for the presence of any HIV genes.

PCR-screening results: Out of a total of 44 PCR samples, 6 conditions yielded PCR products (Table 1, Figures 3 and 4). Genomic DNA samples 1, 2 and 3 displayed PCR products with a molecular weight around 500 bp (Figure 2) annealing temperature of 50 ℃. Similarly, Sample 1 exhibited a PCR product at 54 ℃ and sample 2 exhibited PCR products at 57 ℃ and 58 ℃. Thus, the overall analysis revealed that the PCR products were obtained with a frequency of >13% in the 44 reactions that were performed and analyzed in this study. These PCR products were further sequenced and analyzed to detect the presence of any HIV related genes.

Annealing temperature(˚C)	Sample1	Sample 2	Sample 3	Sample 4
50 ˚C	Detected	Detected	Detected	Not Detected
51 ˚C	Not Detected	Not Detected	Not Detected	Not Detected
52 ˚C	Not Detected	Not Detected	Not Detected	Not Detected
53 ˚C	Not Detected	Not Detected	Not Detected	Not Detected
54 ˚C	Detected	Not Detected	Not Detected	Not Detected
55 ˚C	Not Detected	Not Detected	Not Detected	Not Detected
56 ˚C	Not Detected	Not Detected	Not Detected	Not Detected
57 ˚C	Not Detected	Detected	Not Detected	Not Detected
58 ˚C	Not Detected	Detected	Not Detected	Not Detected
59 ˚C	Not Detected	Not Detected	Not Detected	Not Detected
60 ˚C	Not Detected	Not Detected	Not Detected	Not Detected

Table 1. PCR-screening results for 4 genomic DNA samples.

Sanger sequencing results: The 6 PCR products that were identified by qualitative analysis were sequenced and the chromatograms were analyzed to detect any possible HIV genes. A representative chromatogram is shown in Figure 5.

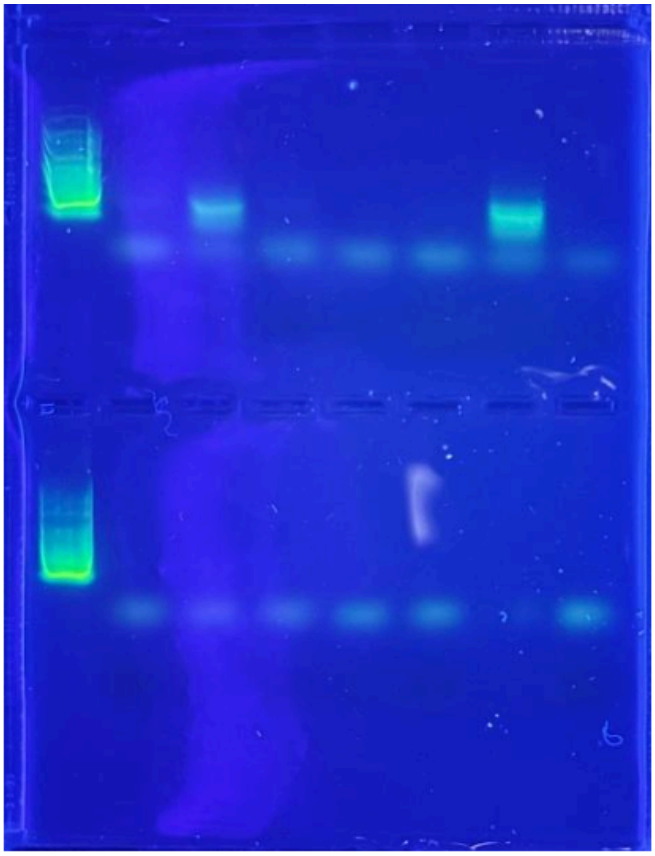

Figure 4. Agarose gel electrophoresis analysis of PCR products.

As shown in Figure 5, the quality of the chromatogram was very low. Too many overlaps between the peaks resulting in a crowded chromatogram is indicative of non-specific primer binding during the Sanger di-deoxy PCR. However, the software was able to perform the base calling. Hence the sequence was further analyzed by performing the NCBI BLASTn search. No homologs were found in the BLASTn search for all 6 sequences. These results indicate that the PCR products that were obtained in certain reactions were non-specific amplicons and/or primer dimers.

Conclusion and Future directions:

In conclusion the present study clearly demonstrates that there were no HIV genes detected from the genomic DNA samples of healthy volunteers that fully recovered from COVID-19 infections. However, the current study was performed on a small sample size and in future bigger sample sizes should be screened in order to confirm the absence of any possible HIV genes in the healthy volunteers that fully recovered from COVID-19 infections. Additionally, it is evident that coronavirus is not a man-made or genetically engineered virus. It evolved and crossed species from bats to the humans.

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Conflict of interest: The authors declare no conflict of interest in this study.

Author contributions: N.P. performed all the wet lab experiments. S.P. co-supervised N.P. R.S.Y. is the principal investigator who designed the project, trained N.P., secured required material for the project, provided the laboratory space and facilities needed. R.S.Y. wrote, edited and finalized the manuscript. All authors approve the final manuscript.

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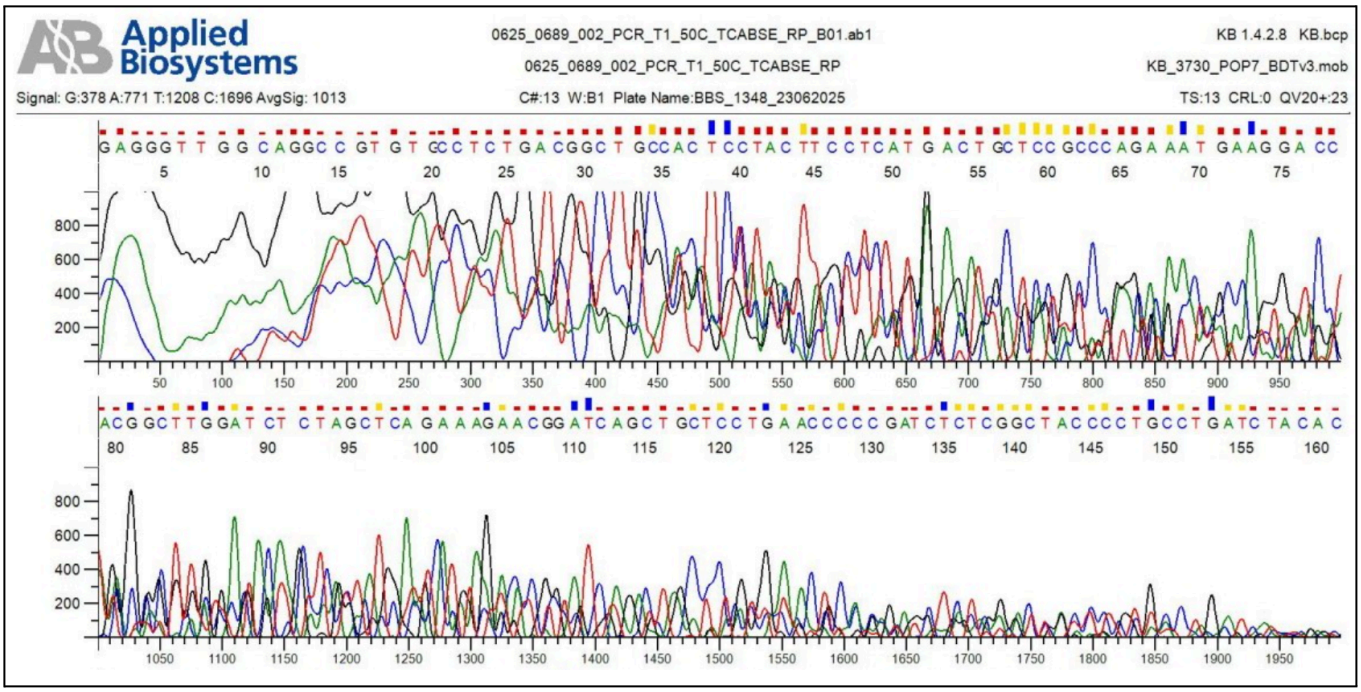

Figure 5. DNA chromatogram result of Sanger sequencing.

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