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Lessons from the Felidae: A Quest to Understand HIV and other Pathogenic Immunodeficiency Viruses

Far from the limelight of the human and mouse genome projects, the Feline Genome Project is striking gold. Researchers at the National Cancer Institute's Laboratory of Genomic Diversity in Frederick, Maryland expect to complete a genetic map far less detailed than the those of the human or mouse, but equally (if not more) important and useful to the study of human genetic diseases. The feline genome has proven to have an organization similar to that of humans[1]. With cats and humans sharing almost 60 inherited diseases, including polycystic kidney disease, diabetes, and certain common immune cell cancers, this genome should develop into a rich resource of genetic diseases that cannot be studied in mice2. Felines are also capable of similar infectious and acquired disease--- including its own version of AIDS, which is induced by feline immunodeficiency virus (FIV)1. Surprisingly, however, the presence of FIV in wild cats does not cause AIDS. Seen in 25 felid species around the world3, this resistance to AIDS means that these cats may hold the key to fighting human deficiency virus (HIV) in humans by providing an excellent model for understanding the evolutionary history of immunodeficiency viruses, the coadaptation of virus and host, and approaches to intervention strategies against lentivirus infections.

Feline immunodeficiency virus was first isolated in 1986 from a domestic cat with AIDS-like symptoms-- an infection of the gut, skin lesions, respiratory tract infection, and wasting4. The owner brought the cat to Niels Pederson's lab at the University of California- Davis. At the

time, the only virus other than HIV known to attack the immune system's T-cells was Simian immunodeficiency virus (SIV), a lentivirus that affects primates such as chimpanzees and monkeys and is an immediate ancestor of HIV3. Pederson discovered the cat to have an immunodeficiency virus of its own, a virus belonging to a class known as retroviruses, which inserts its genetic code into a host's DNA. FIV has been subsequently been found worldwide in different species of felidae. In 1989 Margaret Barr et al. at Cornell reported finding antibodies to FIV in several captive exotic cats and in the wild Florida panthers5. In a search of over 2,000 samples of frozen cat serum, Stephen O'Brien of the National Cancer Institute found that the prevalence of FIV among these was enormous3. Furthermore, it has been reported that these wild members of the family Felidae (i.e., panthers, lions, and bobcats) harbor lentiviruses genetically similar and morphologically analogous to FIV, but do not experience the AIDS-like symptoms6. Thus the discovery of this pathogenic virus in so many different species (25) means first that the virus is not new, and second, because it has not killed off the wild cat hosts, perhaps FIV has evolved over millennia into a symbiosis with its host.

Because the FIV-infected wild cat species are healthy, the question remains as to what differences among the viruses, their hosts, or virus-host interactions have determined this lack of pathogenicity of FIV in various species6. By examining this feline virus, one can draw parallels to SIV and HIV in relation to the delicate balance between pathogenic viruses and their hosts in the outbreak of disease.

Virus-host interaction is a major element in the study of pathogenic viruses and their relationships to one another. In studies of HIV and SIV, it has been demonstrated that variations in the immune response of the host to the virus relate to pathogenicity. For example, unlike humans, chimpanzees that are persistently infected with HIV-1 fail to develop

immunodeficiency, suggesting a difference between the host species' permissiveness to the disease, a factor that depends on how long the host has had to adapt to the virus7. Several strains of SIVsm have been found which are not pathogenic in their natural hosts, sooty mangabey, but cause an AIDS-like disease when in contact with species that have no natural SIV infection, such as Asian macaques7. The same can be seen in FIV, where the virus is currently pathogenic in domestic cats yet non-pathogenic in the wild species. A further parallel will show that HIV, which is currently pathogenic to humans, can someday become non-pathogenic and exist in symbiosis with the human host. This reasoning follows because the FIV sequence variation is lower in domestic cats than in wild species2, illustrating, according to theory, that not enough time has been allowed for variety in this strain of the virus, thus its relatively large virulence. Moreover, HIV-1 has been in the human population for less than 100 years8, and its severity therefore reflects the emergence of a new viral strain upon a large population that has no natural infection. The relative virulence of a pathogenic agent in this way may be determined by the length of time that it has been available for coadaptation of virus and host populations.

On the other hand, other elements can determine relative virulence. SIV studies have also indicated that pathogenicity may be characteristic of specific virus-host combinations. While coadaptation of host and viruses in the African monkey species exists such that SIV infection is widespread, yet non-pathogenic, macaques develop AIDS when infected with some strains of SIV from sooty mangabey and African green monkey, but not from others8. Due to the fact the that these retroviruses mutate rapidly, the results of the macaque study may prove to show that the SIV of the sooty manabey and African green monkey are "newer" strains than the one which the macaque has learned to live with. Thus host selective pressures on viral

pathogenicity are another factor in differing virus-host interactions.

Another lesson to be learned from the comparison of FIV, SIV, and HIV is the determination of the mechanism by which a host species becomes resistant to viral infection. The resistance is often already there among the natural genetic variation present in any population6. It is through selective elimination of susceptible individuals and consequent increased reproductive success of resistant individuals that the trait of resistance emerges and is noted. A study has shown that long-time survivors of macaque SIV infection have higher antibody titers than macaques which succumb to disease earlier6, which suggests that disease resistance correlates with strength of antibody production. Studies of maternal transmission of both HIV-1 and SIV have found a number of healthy seronegative children, born to seropositive mothers, that harbor HIV- infection. It is suggested that exposure of a fetus to HIV-1 could lead to a form of tolerance, in which no antibodies to HIV-1 are produced and no T-cells are destroyed9. This also has occurred in a study of FIV. Healthy seronegative domestic cats that carry FIV nucleic acids have been observed6. Thus this is a possible mechanism by which for example, the wild cats, have developed resistance to FIV pathogenesis6.

While producing stronger antibodies or intentionally exposing a fetus may not be ways in which one could apply to HIV lessons learned from FIV and SIV, but continued studies of these lentiviruses should bring about better ideas as to how resistance can be conferred on the viral host. Further studies include a look into how the viruses are related, answers which lie in the structures of the many different strains of virus from collected and analyzed blood samples.

Once a blood sample is tested for FIV antibodies and is determined to harbor the virus, the DNA of infected white blood cells, where the FIV has inserted its own genes, is extracted. Genetic probes are added in order to find a segment of the virus's polymerase gene, the most

slowly evolving and thus the most informative part of the virus's genome3. Finally, the researchers prepare the segment so that a computer can determine its sequence of base pairs. In analyzing these sequences, one is able to arrange the different strains of FIV into an evolutionary tree according to the theory that different strains which are structurally more closely related have had less time to drift apart. The resulting tree and the evolutionary history contained in it tells the story of how the FIV virus has evolved with the cat and is helping to decipher and genetically link all immunodeficiency viruses.

According to O'Brien's study3, FIV has indeed been with cats for a long time, long enough for them to have evolved their own strains of the virus. O'Brien cannot say with certainty how or when the virus traveled, or "jumped" from one species to another, but judging from distinct differences in each strain's structure (i.e. the lion and puma strains are 25 percent different)3, the date is probably ancient and mechanisms only to be hypothesized, due to incomplete knowledge of other genomes which may have taken part in the transferring of the virus3. (See Appendix for possible scenerios) The jump of a retrovirus from its host to a new species is a rare event, since retroviruses finely attune themselves to their host species' genome. Also there are quite a few conditions which must be favorable in order for FIV, HIV, or SIV to infect the host. Generally, it must find the right cell in the host animal, infect the cell, take over its cellular machinery, replicate, release these copies, and escape the surveillance of the host's immune system so that it can infect another cell. This process, described as a "lockstep" process3, is one in which the virus cannot make a mistake or its host will kill it. Thus, the evidence of many infected cat species, illustrating the jumping from species to species of FIV, brings forth new information about immunodeficiency viruses from which one may be able to

conclude that domestic cats are simply a new species to which FIV has jumped, and that as hosts, they, in a position similar to that of humans with HIV, have yet to adapt to the virus.

The FIV trees give clues about its relationship to other immunodeficiency viruses--- like HIV. HIV is in a special group of retroviruses called lentiviruses-- those which can take years to develop from infection to disease. Lentiviruses have been found in horses, sheep, goats, cattle, old-world monkeys, cats, and humans. Those closest to HIV are SIV, FIV and BIV (bovine immunodeficiency virus). O'Brien's team has compared certain sequences of the polymerase gene in FIV with those in the other three lentiviruses and have devised a scenerio that could explain the variations between them (see Appendix)3.

With the spread of HIV in the world today, it has become necessary to develop model systems relevant to the treatment of lentivirus infections10. The most prominent and feasible non-human primate model is the simian immunodeficiency infection in macaques. Both HIV-1 and SIV use a common receptor, the CD4 T-cell, and the host cell ranges are similar for the two viruses10. However, adult macaques have failed as a useful model for testing drug treatments and the costs of purchasing and housing them are high relative to non-primate models. The chimpanzee primate model is closer to humans, but they are an endangered species and expensive to maintain, making them impractical for extensive preliminary tests.

The smallest and most manipulable natural model, though a nonprimate lentivirus, is FIV. Studies to date have resulted in the cloning and nucleotide sequence analyses of diverse strains of FIV from North America, Europe, and Asia10. These studies have defined the basic structural, enzymological, and regulatory elements of the virus, making FIV favorable for molecular manipulations in detailed studies of the lentivirus life cycle, which could lead to the development of intervention strategies.

In conclusion, African monkeys, lions, and other wild cat species have managed over millennia to come to a peaceful arrangement with simian immunodeficiency virus and feline immunodeficiency virus, respectively. Because of the genetic similarities between these viruses and human immunodeficiency virus, it is hoped that HIV will someday attain the same kind of symbiosis with its human host. Meanwhile, by studying and understanding the evolutionary history of FIV and SIV, and comparing genes involved with disease resistance in wild cats, monkeys and long-term HIV survivors, it is suspected that somewhere in these genomes lies the ultimate cure for AIDS.

Citations:

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4. Pederson NC, Ho EW, Brown ML, Yamamoto JK. 1987. "Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome." *Science* 235: 790-793.
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APPENDIX

Hypothetical scenario involving FIV, SIV, and HIV:

"Several million years ago, perhaps in Africa or the Middle East, the first link was forged in the chain that has led to HIV. An ancestor of today's lions killed and fed on a BIV-infected bovid, such as buffalo. The virus made a devilish twist in the cat--- it figured out how to work the immune system of a new family of mammals and became FIV. Subsequently that ancestral cat passed the disease through biting to its fellow cats; some of them in turn passed it to other cat species. Eventually one of those species bit a monkey, which escaped the attack and survived. This time FIV worked its way into the primate body and became SIV. Some thousands of years later, perhaps because of human's hunting and butchering monkeys, SIV devised a way to infect humans."

--argument by Stephen O'Brien of the National Cancer Institute

FIV evolutionary theory:

FIV may have first entered the felid family roughly 6 million to 3 million years ago[3]. An ancestor of all the wild cats today became infected with the original strain of FIV, and since then, the disease has mutated over and over again, infecting new species with an old, yet changed virus.

SIV evolutionary theory:

SIV first evolved in the African monkeys. Some time ago there was an 'adaptive episode' in which some of the monkeys were resistant to the virus for genetic reasons and they survived the SIV epidemic, while all the others died off. The virus could have also become attenuated and less virulent, or some version of both. --and so there was a standoff between the host and the virus3.

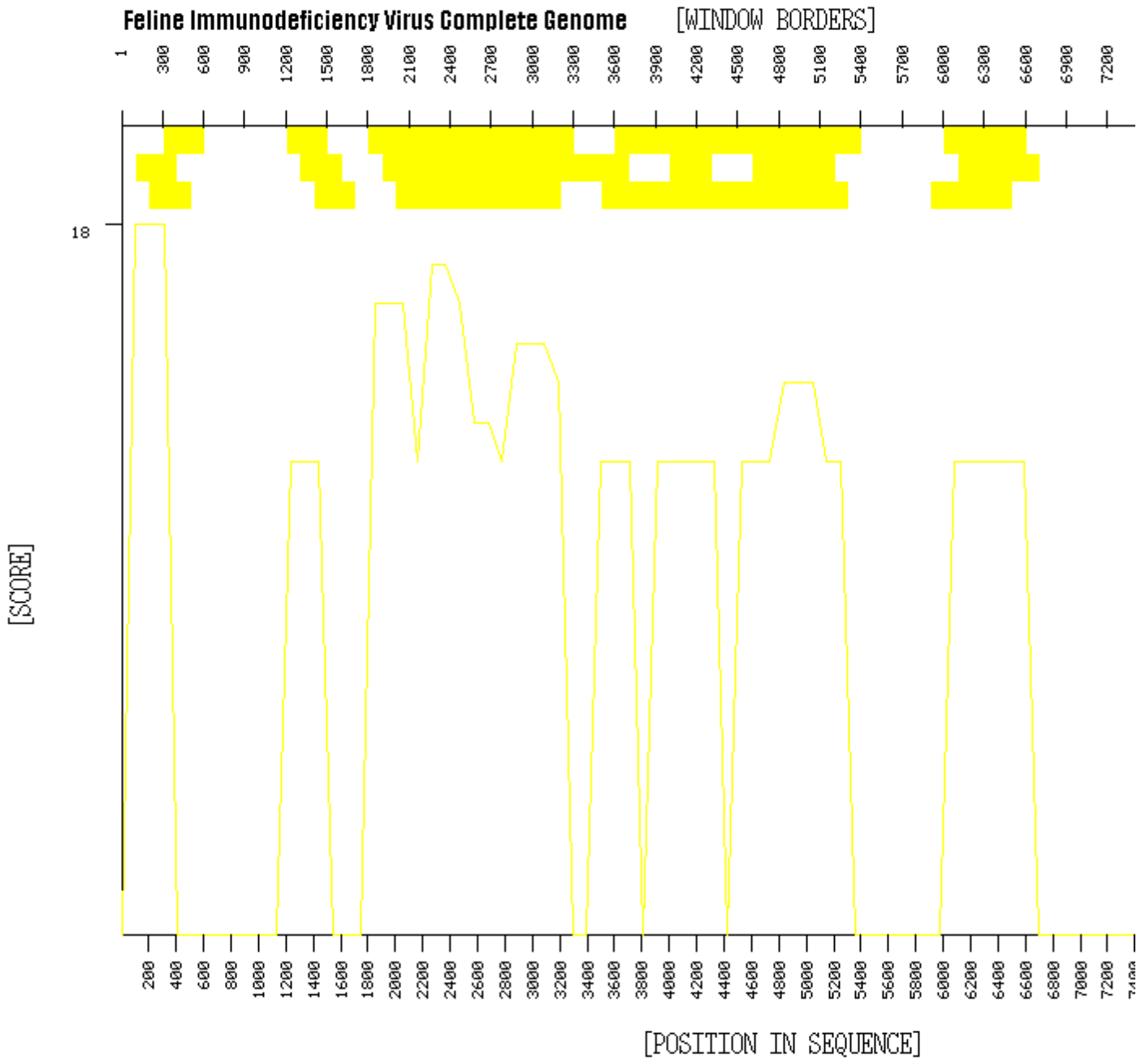
Sequence Searches/ Display of Genetic Data:

Thanks to Professor Brutlag's Genomics and Bioinformatics seminar, I have learned how to perform genetic data searches as well as how to compare this data. Therefore I thought it appropriate to search for and illustrate the research reported in the preceding paper comparing the the genomes of FIV, SIV, and HIV. The FASTA versions of these sequences were obtained through the NCBI website**A** . Alignment and graphs were produced by the genotyping tool on the NCBI-Retrovirus page**B**. This tool uses the BLAST algorithm. The input sequences are compared to a specified reference sequence through the use of "windows", which divide the sequences into a specified number of parts. I used a window size of 300 on all three graphs. In the pages following, I have shown that with HIV as the reference sequence, I have compared HIV to FIV in the first graph and to SIV in next. The last graph illustrates a comparison of FIV and SIV genome sequences.

A <<http://www.ncbi.nlm.nih.gov>>
B <<http://www.ncbi.nlm.nih.gov/retroviruses/subtype/subtype.html
HIV sequence: >ref|NC_001802|gd_HIVHXB2CG Accession # AF033819
FIV sequence: >gi|323933|gb|M25381.1|FIVCG Accession # M25381
SIV sequence: >gi|5106562|gb|AF131870.1|AF131870 Accession # AF131870

Comparative Genomic Organization:

In spite of some sequence divergence, FIV, SIV, and HIV have maintained a similar basic genomic structure. These lentviruses share basic structural features within their gag, pol, and env genes, but vary in other regions (in FIV and HIV substantially in the regions encoding short open reading frames10). Certain structural motifs have remained relatively conserved. Little is known of the specific functions of some of these motifs, but their conservation suggests that they probably have important roles in retrovirus life cycle. Because FIV, SIV, and HIV cause a similar disease syndrome, each of the conserved domains may serve to develop approaches to disrupt virus infection.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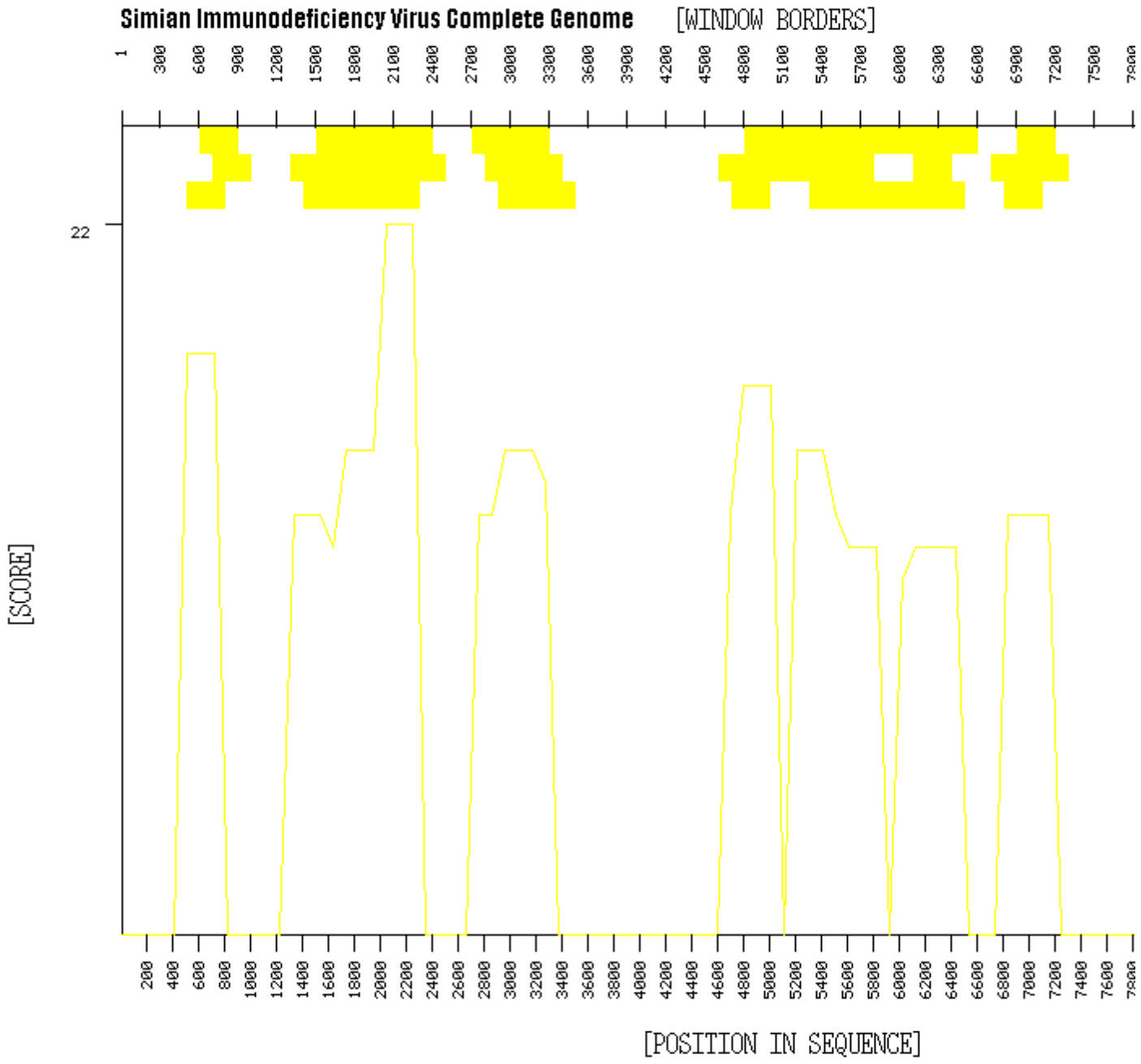
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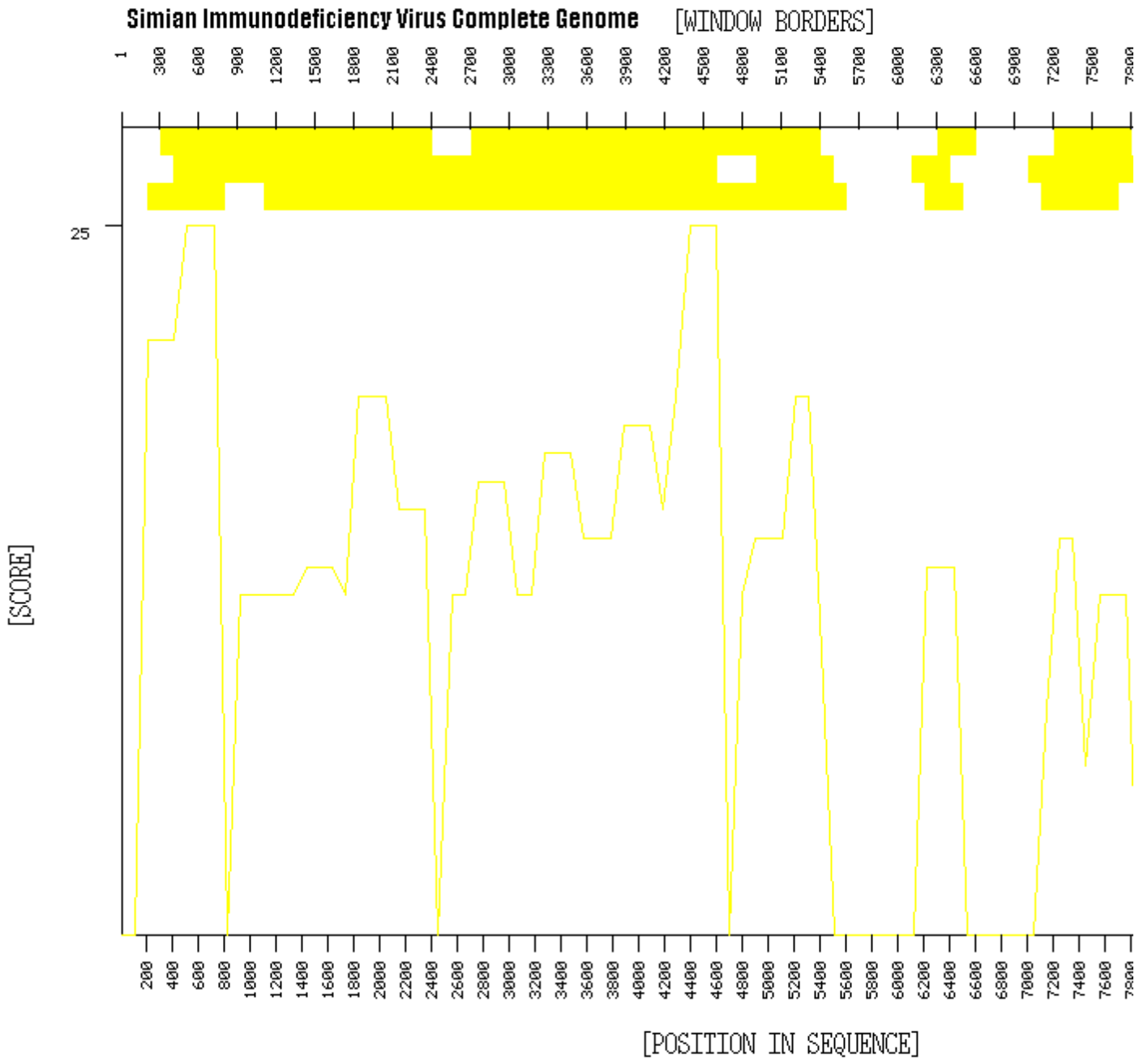
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