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CONSIDERATION OF MULTIPLE TEMPLATE SWITCHES IS CRITICAL TO THE DETERMINATION OF THE RECOMBINATION RATE ON THE HIV GENOME

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Retroviral recombination drives viral diversity and facilitates the emergence of immune escape and drug resistant mutants that contribute to disease progression. Current estimates of retroviral recombination rates are based on indirect measurements that do not take into account the effects of multiple recombination events. In the presence of multiple template switches, any even number of template switches result in no observed recombination and any odd number is detected as a single recombination event. We demonstrate that ignoring multiple recombination events consistently underestimates the true recombination rate, especially over large genetic distances and high rates of recombination. Here, we present a novel approach to measure rates of recombination across different gene segments regardless of the effects of genetic distance and the overall rate of recombination. We apply these tools to a novel HIV-1 marker system, which mimics the recombination process between closely related genomes, analogous to those found within the quasispecies of an infected individual. We directly measure the recombination rate in *gag*, correcting for the effects of multiple template switches and background recombination. Furthermore, our analysis indicates that recombination rates are likely to vary across the viral genome. This system is applicable to other studies to accurately measure the recombination rate that is critical for the diversification of retroviruses.