A Comparison Of Parallel Pyrosequencing And Sanger Clone-based Sequencing And Its Impact On The Characterization Of The Genetic Diversity Of Hiv-1.

Liang, B; Luo, M; Scott-Herridge, J; Semeniuk, C; Mendoza, M; Capina, R; Sheardown, B; Ji, H; Kimani, J; Ball, BT; Van Domselaar, G; Graham, M; Tyler, S; Jones, SJ; Plummer, FA

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Abstract:

BACKGROUND: Pyrosequencing technology has the potential to rapidly sequence HIV-1 viral quasispecies without requiring the traditional approach of cloning. In this study, we investigated the utility of ultra-deep pyrosequencing to characterize genetic diversity of the HIV-1 gag quasispecies and assessed the possible contribution of pyrosequencing technology in studying HIV-1 biology and evolution. METHODOLOGY/PRINCIPAL FINDINGS: HIV-1 gag gene was amplified from 96 patients using nested PCR. The PCR products were cloned and sequenced using capillary based Sanger fluorescent dideoxy termination sequencing. The same PCR products were also directly sequenced using the 454 pyrosequencing technology. The two sequencing methods were evaluated for their ability to characterize quasispecies variation, and to reveal sites under host immune pressure for their putative functional significance. A total of 14,034 variations were identified by 454 pyrosequencing versus 3,632 variations by Sanger clone-based (SCB) sequencing. 11,050 of these variations were detected only by pyrosequencing. These undetected variations were located in the HIV-1 Gag region which is known to contain putative cytotoxic T lymphocyte (CTL) and neutralizing antibody epitopes, and sites related to virus assembly and packaging. Analysis of the positively selected sites derived by the two sequencing methods identified several differences. All of them were located within the CTL epitope regions. CONCLUSIONS/SIGNIFICANCE: Ultra-deep pyrosequencing has proven to be a powerful tool for characterization of HIV-1 genetic diversity with enhanced sensitivity, efficiency, and accuracy. It also improved reliability of downstream evolutionary and functional analysis of HIV-1 quasispecies