LONG-READ SEQUENCING ASSAY ALLOWS ACCURATE CHARACTERIZATION OF THE HIV-1 RESERVOIR

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Laurens Lambrechts[1,2], Rita Verstraeten[1,2], Noah Bonine[1,2], Ytse Noppe[1], Marion Pardons[1], Basiel Cole[1,*], Linos Vandekerckhove[1,*]
[1]HIV Cure Research Center, Ghent University, 9000 Ghent, Belgium, [2]BioBix, Ghent University, 9000 Ghent, Belgium
*These authors contributed equally.

BACKGROUND

- The advent of near full-length (NFL) HIV-1 proviral genome sequencing greatly expanded our understanding of the quality of the viral reservoir.
- However, current NFL assays are based on labor-intensive and costly principles of repeated PCRs at limiting dilution, restricting their scalability. We developed a long-read sequencing assay to characterize **many proviral genomes in parallel from bulk DNA**.

METHODS

- Individual proviruses were tagged with a different unique molecular identifier (UMI) at each end during a single reaction, followed by NFL PCR amplification and long-read sequencing on an Oxford Nanopore MinION (Figure 1). Next, UMI-based demultiplexing allowed for the construction of highly accurate consensus genomes, while excluding aberrant chimeric PCR artefacts.

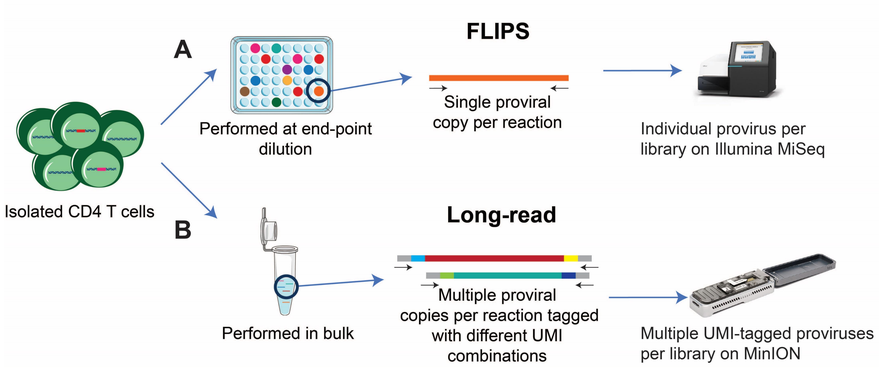

Figure 1. Schematic overview of the used assays. A) The full-length individual proviral sequencing (FLIPS) assay relying on limiting dilution. B) The long-read assay relying on unique molecular identifiers (UMI).

- The assay was performed on 15 chronic ART-suppressed individuals, using a fixed input of 500 ng DNA extracted from peripheral blood CD4 T cells.
- In addition, Full-Length Individual Provirus Sequencing (FLIPS) was performed on 2 individuals. Data from both assays were compared through phylogenetic analyses.

RESULTS

Assay can be used on diverse range of reservoir sizes

- The sensitivity of the long-read assay was determined on a DNA dilution series of J-Lat in uninfected Jurkat revealing the lower limit to be an input of <8 HIV-1 copies/500 ng DNA.
- HIV-1 proviruses were successfully sequenced on a participant cohort with a wide range of reservoir sizes (321-6581 total HIV-1 DNA copies/million CD4 T cells, Figure 2) and performed well on both large and small reservoir sizes.

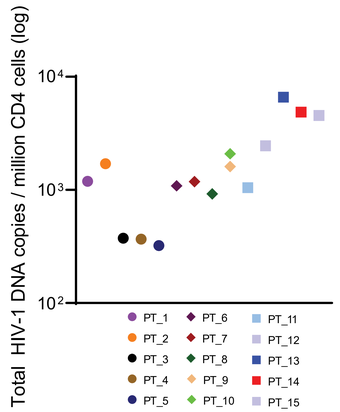

Figure 2. Reservoir sizes of the participant cohort measured by total HIV-1 DNA ddPCR.

Assay allows for HIV-1 genome classification

- The long-read assay yielded an average of 14 distinct HIV-1 proviruses per participant (range: 3-42). Across all participants, 213 distinct proviruses were retrieved and subjected to genome classification of which 8% were found to be genetically-intact (Figure 3).

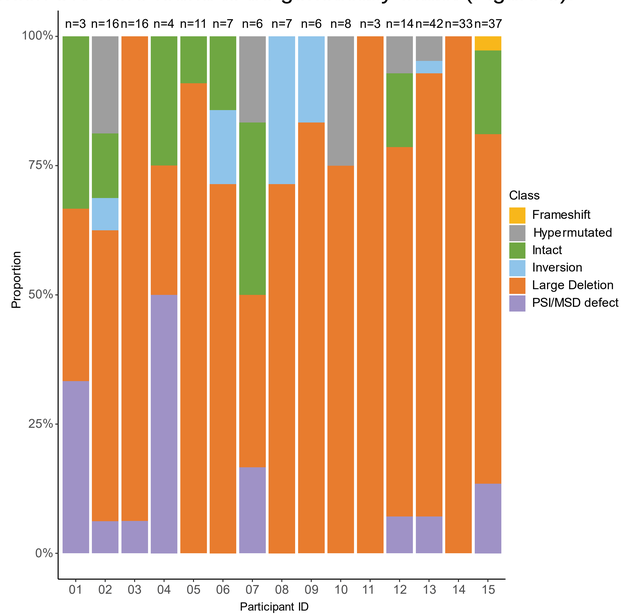

Figure 3. Proportions of the HIV-1 genome classes per participant acquired with the long-read assay. On the top of each bar, the number of generated proviruses per participant are listed. MSD = major splice donor

Overall agreement on observed proviral reservoir composition

- In terms of reservoir composition, data obtained with the FLIPS assay showed an overall agreement with data obtained with the long-read assay.
- In an individual with limited clonality (6% clonality based on FLIPS, n=1 clone) only 1 overlapping provirus was found, while an overlap of 3 proviruses was observed in an individual with higher clonality (91% clonality based on FLIPS, n=4 clones) as seen in Figure 4.
- Comparing these 4 overlapping proviral consensus genomes to their matching FLIPS counterparts showed an average sequence accuracy of 99,97%.

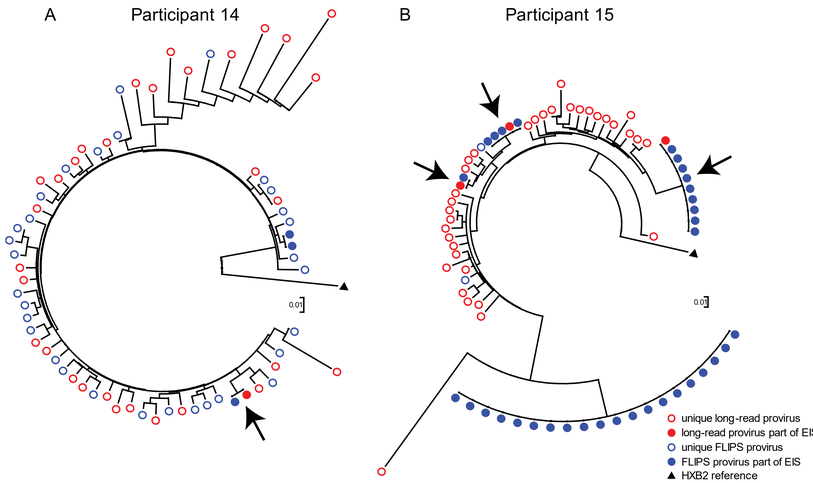

Figure 4. Phylogenetic analysis by Maximum Likelihood Trees for A) Participant 14 and B) Participant 15. Proviruses part of an expansion of identical sequences (EIS) are represented by filled circles. Arrows indicate matches of identical proviruses between the FLIPS and long-read assay.

CONCLUSIONS

- The long-read assay offers a high-throughput NFL sequencing method which enables **an accurate characterization of the proviral landscape while retaining sequencing accuracy** comparable to current gold standard NFL assays.

ADDITIONAL KEY INFORMATION

Contact: Laurens.Lambrechts@ugent.be

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HIV Cure Research Center