Poster # 362

Measurement of Integrated HIV DNA by Pulse-Field Gel Electrophoresis and ddPCR.

Steven M Lada[1,2], Caroline C Ignacio[1], Douglas D Richman[1,3], Matthew C Strain[1,2].
[1]University of California San Diego, La Jolla, California, USA
[2]Veterans Medical Research Foundation, San Diego, California, USA
[3]Veterans Affairs San Diego Healthcare System, San Diego, California, USA

Steven M. Lada
University of California San Diego
9500 Gilman Drive MC 0679
La Jolla, CA 92093-0679, USA
Tel: (858) 642-1106
Fax: (858) 552-7445
E-mail: slada@vapop.ucsd.edu

Background

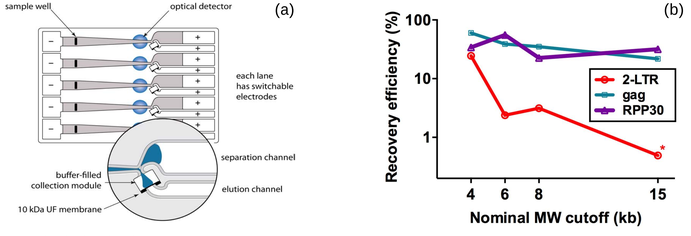
- A latent reservoir of chromosomally-integrated HIV DNA persists in long-lived CD4+ T cells during antiretroviral therapy (ART) and must be eliminated or controlled to achieve a sterilizing cure.
- Droplet digital PCR (ddPCR) allows precise and sensitive measurement of HIV DNA but does not distinguish between integrated and unintegrated molecular forms.[1]
- Alu-Gag PCR[2] and physical separation of high molecular weight (HMW) DNA by gel electrophoresis[3] are established methods that specifically measure integrated HIV DNA but are cumbersome and require large amounts of starting DNA material, limiting their application in translational studies.
- Pulsed-field gel electrophoresis (PFGE) uses time-varying electric fields to isolate nucleic acids, and can achieve higher molecular-weight specificity, particularly for large DNA fragments.

Objective

- We sought to develop a scalable method to selectively measure integrated HIV DNA with high sensitivity and precision.

Methods

- DNA was extracted from cell pellets with Qiagen QIAamp DNA Blood kit.
- High molecular weight DNA was separated by Pulse-field Gel Electrophoresis (PFGE) with the BluePippin cassette system. (a)
  - 0.75% agarose pre-cast cassettes; ~5 hour run time
  - Process parameters were optimized to maximize selective recovery of HMW DNA. (b)
- HIV Gag, HIV 2-LTR junction, and host cell RPP30 were measured by droplet digital PCR (ddPCR).[1,3]
- Cell samples analyzed included U1 cell line, CD4+ T cells infected in-vitro, 8 ART-naïve participants, and 1 ART-suppressed participant.



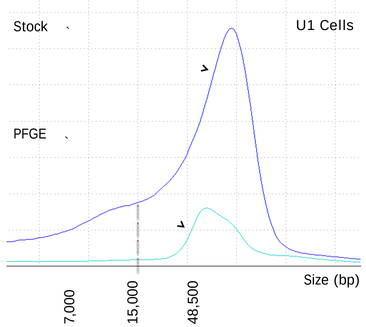
➢ DNA separation in the BluePippin PFGE cassette system.
(a) Samples are loaded into four physically-isolated channels. Migration of dye-labeled size standards in a fifth lane is used to time the gating of a switch that redirects flow into the elution channel. Electrical pulse sequences defined by manufacturer protocols use a series of voltage ramps to maximize resolution near a target molecular-weight cutoff.
(b) Optimal resolution of high molecular weight DNA versus episomal DNA was obtained with the High-Pass 15-20kb sequence, using a nominal collection cutoff of 15kb. *Below limit of detection.

References

1. Strain MC, Lada SM, Luong T, et al. Highly Precise Measurement of HIV DNA by Droplet Digital PCR. *PLoS ONE.* 2013;8(4):e55943. doi:10.1371/journal.pone.0055943.
2. Liszewski MK, Yu JJ, O'Doherty U. Detecting HIV-1 integration by repetitive-sampling *Alu-gag* PCR. *Methods.* 2009;47(4):254-260. doi:10.1016/j.ymeth.2009.01.002.
3. Koelsch KK, Liu L, Haubrich R, May S, Havlir D, Günthard HF, Ignacio CC, Campos-Soto P, Little SJ, Shafer R, Robbins GK, D'Aquila RT, Kawano Y, Young K, Dao P, Spina CA, Richman DD, Wong JK. Dynamics of total, linear nonintegrated, and integrated HIV-1 DNA in vivo and in vitro. *J Infect Dis.* 2008 Feb 1;197(3):411-9. doi: 10.1086/525283.

Results

Figure 1 – PFGE Removes Lower Molecular Weight HIV DNA Species



➢ QC by Agilent 2200 Genomic DNA ScreenTape
Tapestation electropherogram of samples before (Stock) and after (PFGE) extraction. U1 latent HIV cell line is defective in Tat protein but retains all other viral machinery.

Figure 3 – Removal of 2-LTR Circles from PBMC DNA of HIV Infected Subjects

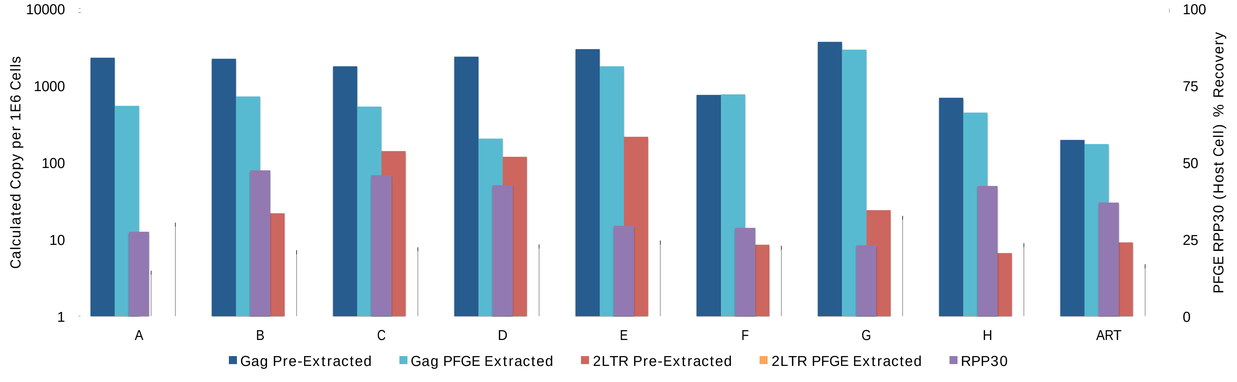
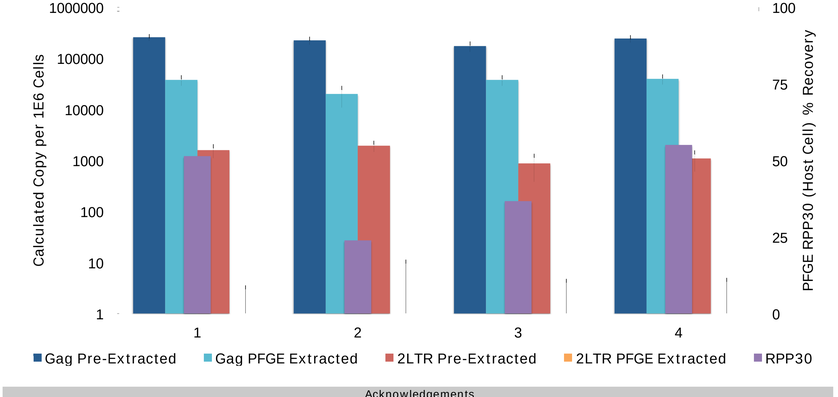


Figure 2 – Reproducible Recovery of Genomic DNA



- Across technical replicates, there was an average of 43% recovery of genomic DNA as measured by RPP30 ddPCR. (Fig. 2) 2-LTR circle HIV DNA was undetectable by ddPCR in all technical replicates of PFGE (Fig. 2) Estimate 99.6% reduction of 2-LTR circle HIV DNA, as determined by calculated limit of detection in PFGE extracted samples. (Fig. 2)
- Our ddPCR assay is able to detect total HIV Gag DNA and episomal contaminant HIV 2-LTR circles but currently cannot uniquely detect 1-LTR circles or linear unintegrated DNA. NL43 CD4 test sample is estimated to ha ve 15% integrated HIV as measured by Gag and removal of contaminant HIV DNA species by PFGE. (Fig. 2)
- PFGE is useful in primary clinical samples to effectively remove HIV 2-LTR circles and other contaminant HIV DNA species, thereby enriching genomic integrated HIV DNA, to improve accuracy of quantitation of the latent HIV DNA reservoir. (Fig. 3)

Conclusions

- With a 15kb cutoff for PFGE, low molecular weight HIV species, such as 2-LTR circles and linear unintegrated, are greatly removed from high molecular weight integrated HIV DNA.
- Using PFGE to remove episomal and linear species of HIV DNA permits the recovery of chromosomal HIV DNA with high purity and efficiency.
- This method provides a sensitive and precise approach to the measurement of integrated HIV DNA with sufficient throughput for translational research studies.

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