HIV TEM-SEQ: TARGETED ENZYMATIC METHYLATION SEQUENCING OF HIV-1 PROVIRUSES

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Background:

- DNA methylation (DNAm) has emerged as a potential mechanism to induce a state of deep latency in '*Block and Lock*" HIV curative strategies. Retroviral transcriptional silencing is associated with methylation of viral promoters; however, knowledge of HIV-1 proviral methylation during latency is controversial due to technical challenges of traditional bisulfite sequencing methods.
- Here, we developed and demonstrate the efficacy of an HIV targeted sequencing method (TEM-Seq) of proviral CpG methylation in latency cell-line models of HIV and in CD4 T cells from a person living with HIV (PWH).

Methods:

- J-Lat 5A8, J-Lat 8.4, ACH-2, and U1 latency cell lines were cultured with DMSO or latency reversal agents PMA: ionomycin, anti-CD3/CD28, or TNF-α. CD4 T cells were positively selected from peripheral blood mononuclear cells (PBMC) collected from a PWH before and after ART.

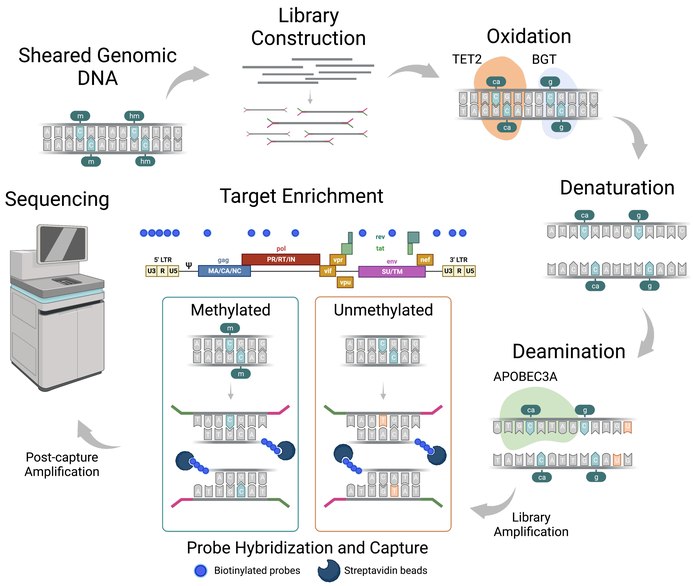


Figure 1: Schema of HIV Targeted Enzymatic Methylation Sequencing (TEM-Seq). Oxidation using TET2 and BGT converts 5mC (m) and 5hmC (hm) to 5-caC (ca) and βg5-hmC (g) products protected from deamination by APOBEC3A. Unmethylated cytosines are converted to uracil allowing identification of methylated residues. Biotinylated oligo probes were designed to target both methylated and unmethylated CpG's of the HBX2 reference genome and autologous HIV-1 sequences. Hybridized methylation targets are captured using streptavidin beads and sequenced on the Illumina NovaSeq platform.

Non-CpG (CHH) HIV-1 proviral methylation is increased compared to CpG methylation in blood CD4 T cells for a PWH prior to ART (ART naïve) and post ART.

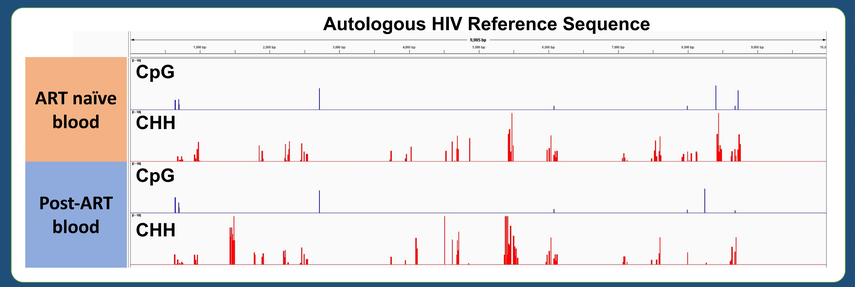
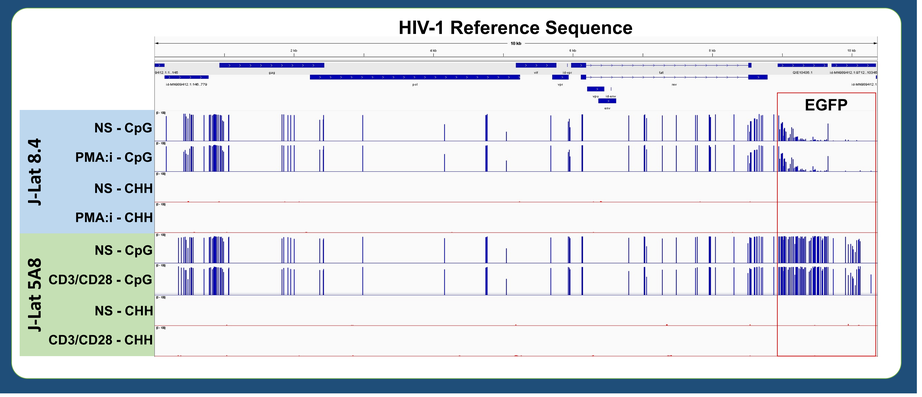


Figure 2: IGV plot of coverage percent CG and CHH DNA methylation. Targeted methylation sequencing identified CpG (blue) and CHH (red) methylation. TEM-seq reads were mapped to autologous HIV-1 sequences using Bismark Aligner.

High levels of CpG methylation observed on J-Lat 8.4 and 5A8 HIV-1 provirus.

Figure 3: HIV TEM-seq of J-Lat cell line reactivation. J-Lat cell line were treated with LRAs or DMSO for 36 hrs. CpG and CHH methylation reads were mapped to J-Lat HIV-1 reference sequence. CpG methylation was increased compared to CHH methylation in J-Lat cell lines.



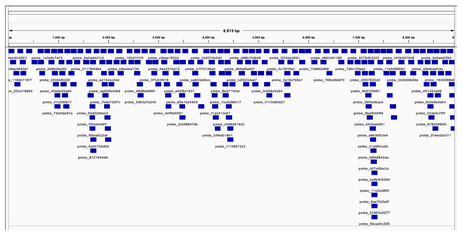


Figure 4: HIV TEM-seq methylation probes for autologous sequence from participant in fig 1.

Figure 5: HIV TEM-seq CpG methylation coverage. J-Lat 8.4 HIV proviral DNA methylation. TEM-seq provides scalable coverage of all HIV-1 proviral CpG sites.

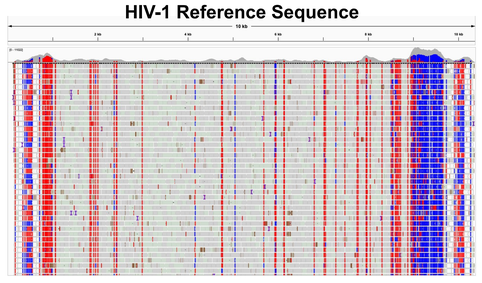
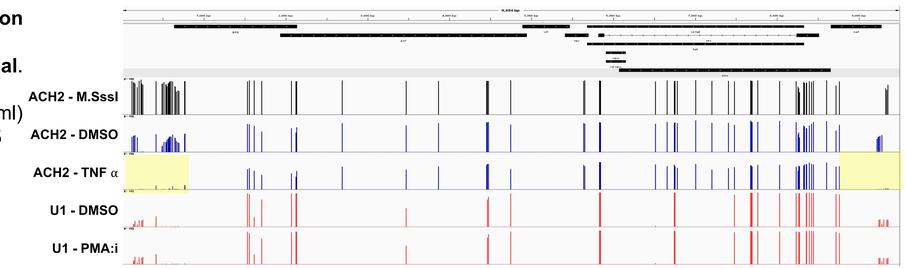


Figure 6: CpG methylation of ACH2 and U1 HIV following latency reversal. ACH2 and U1 cells were treated with TNF-α (5 pg/ml) and PMA/ionomycin (40.5 nM/ 669.5 nM) for 48 hrs. ACH2 DNA was fully methylated using M.SssI methyltransferase as a positive control.



Results:

- Probes were designed spanning the entire HIV-1 genome of autologous sequences allowing full coverage of proviral DNA methylation.
- Autologous viral sequences were referenced in the methylation panel to address viral sequence diversity in primary blood specimens.
- Increased CHH methylation was detected at proviral sequences in primary ART naïve and post-ART CD4 T cells. 99.5% non-CpG conversion rate was measured using control unmethylated lambda DNA (data not shown), indicating this was not a conversion failure.
- Distinct CpG methylation patterns were observed across latency cell lines. CpG methylation was decreased at the 5' LTR following HIV reactivation by LRA's. J-Lat cell lines lacked CHH methylation of the HIV provirus.

Conclusion:

- HIV TEM-Seq provides scalable coverage of all CpG methylation in both primary blood cells harboring HIV including HIV latency cell line models and can be utilized to quantify proviral methylation in *'Block and Lock'* HIV curative interventional strategies.
- We observed a high degree of CpG methylation of the HIV provirus in all latency cell lines tested. Demethylation of the 5' LTR following latency reversal was consistent with previous reports and further supports epigenetic regulation of HIV proviral silencing during latency.
- CHH, but not CpG methylation, was increased in primary immune cells before and after ART initiation. The lack of CpG methylation at the 5' LTR may indicate residual transcription. Further, studies decrypting the relationship of both CpG and CHH methylation and residual HIV transcription *in vivo* are warranted.

Acknowledgements:

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