# PROVIRAL INTEGRATION SITE MAPPING SEQUENCING (PRISM-SEQ) FOR DECTECTION OF HIV-1 INTEGRATION SITES

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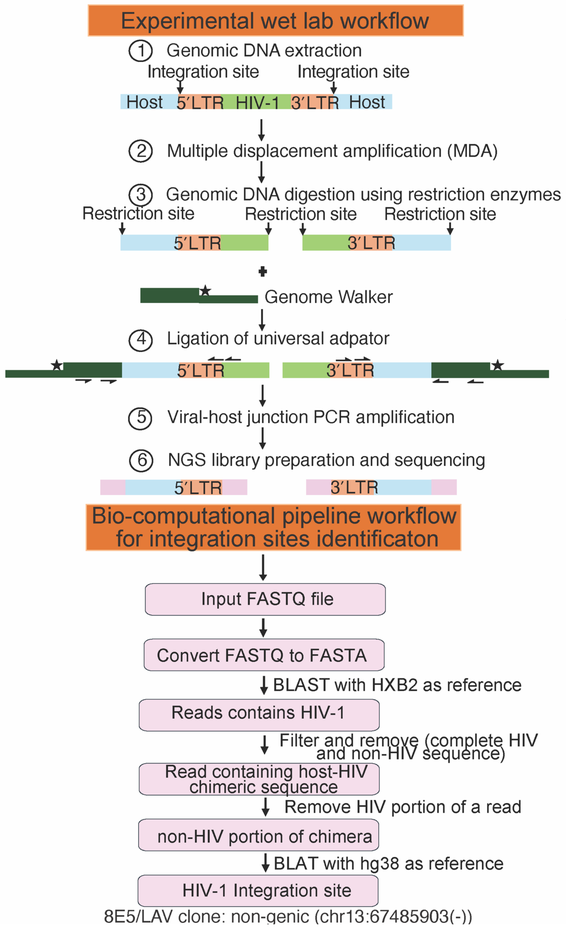
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## BACKGROUND

- Precise detection of HIV-1 integration sites (IS) in the human genome is crucial for studying and characterizing latent reservoirs in people living with HIV-1 (PLWH).
- Lack of ultra-sensitive and economical method to profile proviral integration landscape and longitudinally track infected clones *in vivo* in people living with HIV-1 (PLWH) under ART.

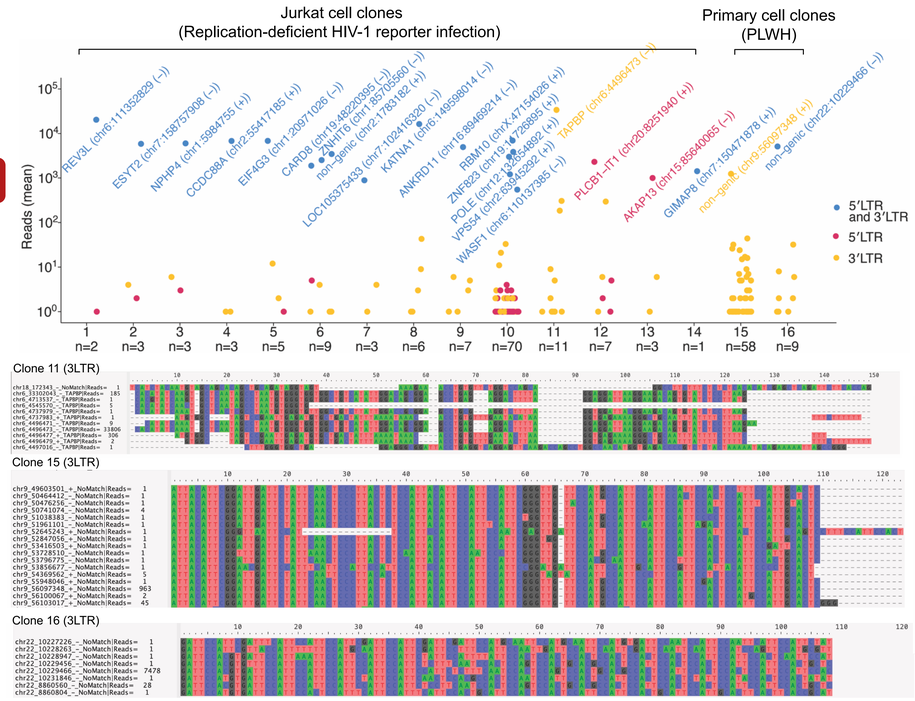
## METHODS

- Isolation of genomic DNA (QIAGEN DNeasy kit) from CD4+ T cells isolated from PLWH under ART or single cell clones and ddPCR-based estimation of proviral DNA copies.
- Input of 50 proviral copies containing genomic DNA, whole genome amplification, and amplification of 5′ and 3′ LTR viral-host junction using Ligation-mediated PCR (LM-PCR).
- PCR amplicon sequencing and bioinformatic analysis using BulkIntSiteR to identify chromosomal coordinates of HIV-1 integration in the human genome.



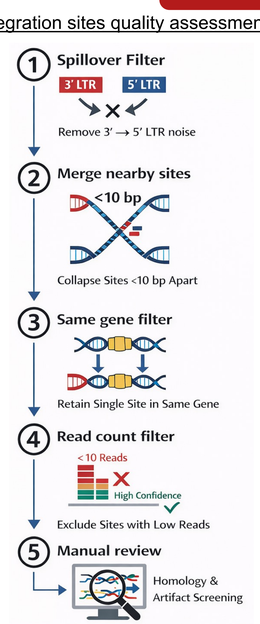

## RESULTS

### PRISM-seq validation on HIV-1 infected CD4⁺ T cell clones




**PRISM-seq recovers 100% single copy integration sites after seven replicates (pool2)**

Integration sites quality assessment




Pool 1 post-filter

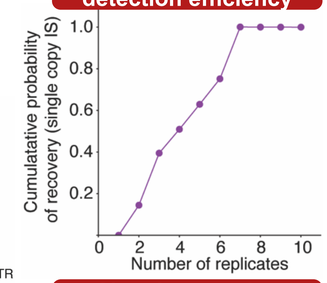
| Clone ID | High-confidence integration site | Average copies per replicate | Number of replicates | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 1 | REV3L (chr6:111352829(-)) | 40 | TRUE | TRUE | TRUE | TRUE | TRUE | TRUE | TRUE | TRUE | TRUE | TRUE |
| 2 | ESYT2 (chr7:158757908(-)) | 1 | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | TRUE | TRUE |
| 3 | NPHP4 (chr1:5984755(+)) | 1 | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE |
| 4 | CCDC88A (chr2:55417185(+)) | 1 | FALSE | FALSE | FALSE | FALSE | FALSE | TRUE | FALSE | TRUE | FALSE | FALSE |
| 5 | EIF4G3 (chr1:20971026(-)) | 1 | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE |
| 6 | CARD8 (chr19:48220395(-)) | 1 | FALSE | FALSE | FALSE | TRUE | FALSE | FALSE | FALSE | FALSE | FALSE | TRUE |
| 6 | ZNHIT6 (chr1:85705560(-)) | 1 | TRUE | TRUE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | TRUE | FALSE |
| 6 | non-genic (chr2:1783182(+)) | 1 | FALSE | FALSE | FALSE | TRUE | FALSE | TRUE | FALSE | TRUE | TRUE | TRUE |
| 7 | LOC105375433 (chr7:102416320(-)) | 1 | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE |
| 8 | KATNA1 (chr6:149598014(-)) | 1 | FALSE | FALSE | TRUE | FALSE | TRUE | FALSE | FALSE | FALSE | FALSE | FALSE |
| 9 | ANKRD11 (chr16:89469214(-)) | 1 | TRUE | TRUE | FALSE | FALSE | FALSE | FALSE | TRUE | FALSE | FALSE | FALSE |
| 10 | POLE (chr12:132654892(+)) | 1 | FALSE | FALSE | TRUE | TRUE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE |
| 10 | RBM10 (chrX:47154026(+)) | 1 | TRUE | FALSE | FALSE | FALSE | FALSE | FALSE | TRUE | TRUE | FALSE | FALSE |
| 10 | VPS54 (chr2:63945292(+)) | 1 | TRUE | FALSE | TRUE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | TRUE |
| 10 | WASF1 (chr6:110137385(-)) | 1 | FALSE | TRUE | FALSE | FALSE | TRUE | FALSE | FALSE | FALSE | FALSE | FALSE |
| 10 | ZNF823 (chr19:11726895(+)) | 1 | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | TRUE | FALSE | FALSE |
| 11 | TAPBP (chr6:4496473(-)) | 1 | TRUE | TRUE | TRUE | TRUE | FALSE | TRUE | FALSE | FALSE | FALSE | TRUE |

Pool 2 post-filter

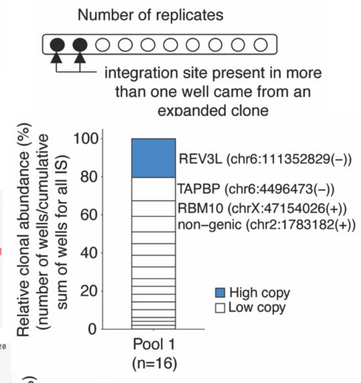
| Clone ID | High-confidence integration site | Average copies per replicate | Number of replicates | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 1 | REV3L (chr6:111352829(-)) | 10 | TRUE | TRUE | TRUE | TRUE | TRUE | TRUE | TRUE | FALSE | FALSE | FALSE |
| 2 | ESYT2 (chr7:158757908(-)) | 10 | TRUE | TRUE | TRUE | TRUE | TRUE | TRUE | TRUE | TRUE | FALSE | TRUE |
| 3 | NPHP4 (chr1:5984755(+)) | 10 | TRUE | TRUE | TRUE | TRUE | TRUE | TRUE | TRUE | TRUE | TRUE | TRUE |
| 4 | CCDC88A (chr2:55417185(+)) | 10 | TRUE | TRUE | TRUE | TRUE | TRUE | TRUE | TRUE | FALSE | TRUE | FALSE |
| 5 | EIF4G3 (chr1:20971026(-)) | 1 | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | TRUE | FALSE | FALSE | TRUE |
| 6 | CARD8 (chr19:48220395(-)) | 1 | TRUE | FALSE | FALSE | TRUE | FALSE | TRUE | TRUE | FALSE | FALSE | FALSE |
| 6 | ZNHIT6 (chr1:85705560(-)) | 1 | FALSE | FALSE | FALSE | FALSE | FALSE | TRUE | FALSE | FALSE | FALSE | FALSE |
| 6 | non-genic (chr2:1783182(+)) | 1 | FALSE | FALSE | FALSE | TRUE | TRUE | FALSE | TRUE | FALSE | TRUE | TRUE |
| 7 | LOC105375433 (chr7:102416320(-)) | 1 | FALSE | FALSE | FALSE | TRUE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE |
| 8 | KATNA1 (chr6:149598014(-)) | 1 | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | TRUE | FALSE | TRUE | TRUE |
| 9 | ANKRD11 (chr16:89469214(-)) | 1 | FALSE | FALSE | FALSE | FALSE | FALSE | TRUE | TRUE | FALSE | FALSE | FALSE |
| 10 | POLE (chr12:132654892(+)) | 1 | FALSE | FALSE | TRUE | FALSE | FALSE | FALSE | TRUE | FALSE | FALSE | FALSE |
| 10 | RBM10 (chrX:47154026(+)) | 1 | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE |
| 10 | VPS54 (chr2:63945292(+)) | 1 | FALSE | FALSE | TRUE | FALSE | TRUE | FALSE | FALSE | FALSE | TRUE | FALSE |
| 10 | WASF1 (chr6:110137385(-)) | 1 | FALSE | FALSE | FALSE | FALSE | TRUE | FALSE | FALSE | FALSE | FALSE | FALSE |
| 10 | ZNF823 (chr19:11726895(+)) | 1 | FALSE | TRUE | TRUE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE |
| 11 | TAPBP (chr6:4496473(-)) | 1 | FALSE | FALSE | TRUE | FALSE | FALSE | TRUE | FALSE | FALSE | FALSE | FALSE |
| 12 | PLCB1-IT1 (chr20:8251940(+)) | 1 | FALSE | FALSE | FALSE | FALSE | TRUE | FALSE | FALSE | FALSE | FALSE | TRUE |
| 13 | AKAP13 (chr15:85640065(-)) | 1 | FALSE | TRUE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | TRUE |
| 14 | GIMAP8 (chr7:150471878(+)) | 1 | FALSE | FALSE | TRUE | FALSE | FALSE | FALSE | TRUE | FALSE | FALSE | FALSE |

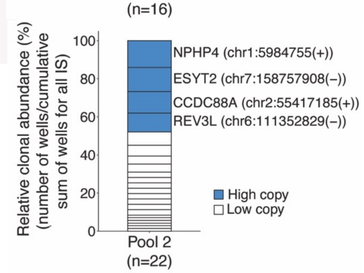
5′LTR and 3′LTR  5′LTR  3′LTR

### PRISM-seq detection efficiency

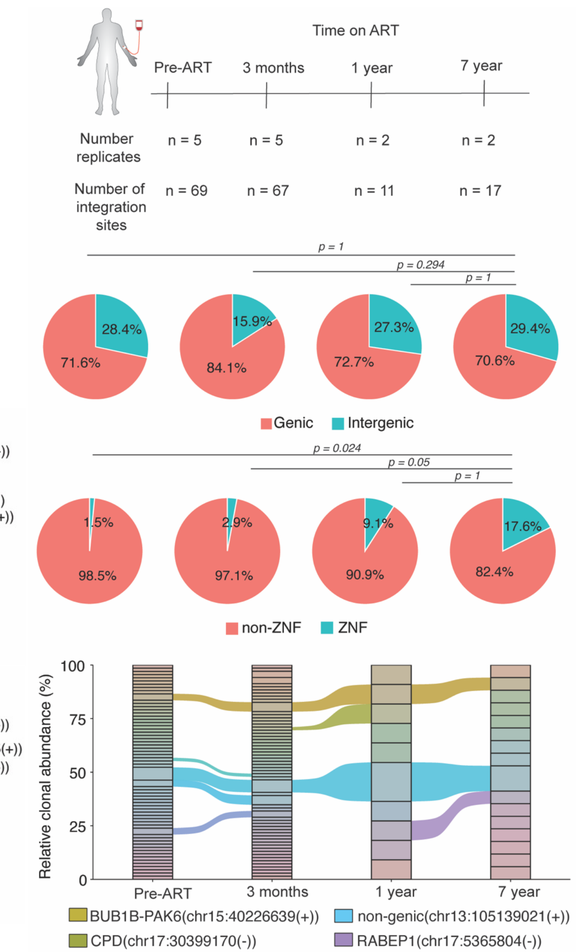



### Clonal expansion estimates




### PRISM-seq application to longitudinal clinical samples from a PLWH




## CONCLUSIONS

- Development of an ultra-sensitive single-molecule workflow for genome-wide recovery of HIV-1 integration sites.
- PRISM-seq enables recovery of integration sites from single-molecule templates within bulk input samples and is compatible with clinical specimens.
- PRISM-seq supports ultra-low-input integration-site analysis and is compatible with limited cell populations, including flow cytometry-sorted HIV-infected cells.
- PRISM-seq does not evaluate proviral genome intactness.

## ACKNOWLEDGEMENTS

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