Integrated Human Immunodeficiency Virus Type 1 Sequence in J-Lat 10.6

Cheng-Han Chung,a,b Anthony R. Mele,a,b Alexander G. Allen,a,b Robert Costello,a,b Will Dampier,a,b Michael R. Nonnemacher,a,b,c Brian Wigdahl,b,c,d

aDepartment of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, Pennsylvania, USA
bCenter for Molecular Virology and Translational Neuroscience, Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, Pennsylvania, USA
cCenter for Clinical and Translational Medicine, Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, Pennsylvania, USA
dSidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania, USA

Cheng-Han Chung and Anthony R. Mele contributed equally to this work. The authors are listed in alphabetical order.

ABSTRACT The full length of HIV/R7/E^-/GFP integrated in the J-Lat 10.6 cell line was sequenced in this study. The single copy of the integrated virus, including the breakpoints from the human chromosome to the provirus, was amplified by two separate PCRs. A 10,200-bp genome sequence was acquired, analyzed, and deposited in GenBank.

The J-Lat 10.6 cell line is a subclone derived from Jurkat-based cells infected with a pseudotyped human immunodeficiency virus type 1 (HIV-1) (genus Lentivirus, family Retroviridae) strain, HIV/R7/E^-/GFP (1, 2). The integrated HIV-1 copy in this cell line is located in the second intron of SEC16A (chromosome 9, position 136468579), providing a useful cell line for studying HIV-1 latency (3). In order to use J-Lat 10.6 for anti-HIV-1 gene editing and design strategies using the clustered regularly interspaced short palindromic repeats (CRISPR) system, it is necessary to have the full proviral DNA sequence (4–11). However, the full-length sequence of integrated HIV/R7/E^-/GFP has not been reported. Here, we amplified two overlapping fragments and performed subsequent Sanger sequencing to acquire the whole genome of HIV/R7/E^-/GFP.

Genomic DNA was isolated from J-Lat 10.6 cells utilizing the QIAamp DNA minikit (catalog number 51304; Qiagen) as described by the manufacturer. In order to determine the HIV-1 proviral sequence, the DNA was amplified as two fragments using newly designed primers (Table 1). The amplicon starting at the 5’ end of the provirus (5’ amplicon) was 8,999 bp and was amplified using primers based on the reported integration site (3); primers 10.6_up5LTR_F and eGFP-N (ReadyMade Primers, catalog number 51-01-05-05; Integrated DNA Technologies), directed to the N terminus of the gene for enhanced green fluorescent protein (eGFP), which replaces nef in this molecular clone, were used for the 5’ amplicon PCR. An adapted single-genome amplification protocol (12) using Platinum Taq polymerase (catalog number 10966026; Invitrogen) was implemented.

The 3’ amplicon encompassed eGFP and the 3’ long terminal repeat (LTR) and was 2,140 bp. It was amplified using primers Frag-26-R-RC and 10.6_down3LTR_R, with the PCR conditions listed in Table 1. The PCR products were enzymatically purified utilizing ExoSAP-IT PCR product cleanup reagent (catalog number 78201.1.ML; Thermo Fisher Scientific). Sanger sequencing was performed by GENEWIZ, Inc. (South Plainfield, NJ), using Applied Biosystems BigDye version 3.1 and the primers listed in Table 1. The reactions were run on an Applied Biosystems 3730xl DNA analyzer.
Quality control of the sequence was performed by end trimming using average quality scores of >16 over 21 bp, followed by assembly with default settings in DNASTAR SeqMan (13). The entire HIV-1 proviral genome reported was 10,200 bp, with a GC content of 43.7%. Every nucleotide within HIV/R7/E/H11002/GFP was sequenced at least twice for a high level of accuracy and was annotated by DNASTAR SeqBuilder for GenBank submission (13). Previously reported mutations in vpr and env and the resulting immature proteins were annotated in the GenBank file (2, 14). There is an insertion of thymine and adenine at nucleotide position 6548, which causes a frameshift of env and an early stop codon at amino acid residue 85. The vpr coding region has an insertion of thymine at nucleotide position 5919, which causes a frameshift of vpr and an early stop codon at Vpr amino acid residue 79.

**Data availability.** The accession number for the genome sequence of HIV/R7/E/H11002/GFP and the flanking integration site is MN989412.

**ACKNOWLEDGMENTS**

This work was funded in part by the Public Health Service, National Institutes of Health, through (i) National Institute of Mental Health (NIMH) grant R01 MH110360.
(B.W., contact principal investigator [PI]), (ii) NIMH Comprehensive NeuroAIDS Center (CNAC) grant P30 MH092177 (Kamel Khalili, PI; B.W., PI of the Drexel subcontract involving the Clinical and Translational Research Support Core), (iii) a Developmental Funding Award (W.D., PI), (iv) National Institute of Neurological Disorders and Stroke (NINDS) grant R01 NS089435 (PI, M.R.N.), and (v) Ruth L. Kirschstein National Research Service Award T32 MH079785 (B.W., PI of the Drexel subcontract involving the Clinical and Translational Research Support Core), (vi) NIMH Comprehensive NeuroAIDS Center (CNAC) grant P30 MH092177 (Kamel Khalili, PI; B.W., PI of the Drexel subcontract involving the Clinical and Translational Research Support Core), (vii) a Developmental Funding Award (W.D., PI), (viii) National Institute of Neurological Disorders and Stroke (NINDS) grant R01 NS089435 (PI, M.R.N.), and (ix) Ruth L. Kirschstein National Research Service Award T32 MH079785 (B.W., PI of the Drexel subcontract involving the Clinical and Translational Research Support Core). The contents of the paper are solely the responsibility of the authors and do not necessarily represent the official views of the NIH. A.G.A. was also supported by the Drexel University College of Medicine Dean’s Fellowship for Excellence in Collaborative or Themed Research (A.G.A., fellow; B.W., mentor).

The following reagent was obtained through the NIH AIDS Reagent Program: J-Lat 10.6 (full-length) cells from Eric Verdin (2).

C.-H.C., A.R.M., and A.G.A. conceived and designed the study. R.C. isolated genomic DNA from J-Lat 10.6 cells. A.R.M. optimized and performed the amplification of the provirus. C.-H.C., A.R.M., and W.D. designed and performed the bioinformatic and statistical analyses. C.-H.C., A.R.M., A.G.A., R.C., W.D., M.R.N., and B.W. drafted the manuscript. All the authors read and approved the final manuscript.

REFERENCES

1. Bieniasz PD, Cullen BR. 2000. Multiple blocks to human immunodeficiency virus type 1 replication in rodent cells. J Virol 74:9868 – 9877. https://doi.org/10.1128/jvi.74.21.9868-9877.2000.

2. Jordan A, Bisgrove D, Verdin E. 2003. HIV reproducibly establishes a latent infection after acute infection of T cells in vitro. EMBO J 22:1868–1877. https://doi.org/10.1093/emboj/cdg188.

3. Symons J, Chopra A, Malatinkova E, De Spiegelaere W, Leary S, Cooper D, Abana CO, Rhodes A, Rezaei SD, Vandekerckhove L, Mallal S, Lewin SR, Cameron PJ. 2017. HIV integration sites in latently infected cell lines: evidence of ongoing replication. Retrovirology 14:2. https://doi.org/10.1186/s12977-017-0011-4.

4. Hu W, Kaminski R, Yang F, Zhang Y, Cosentino L, Li F, Luo B, Alvarez-Carbonell D, Garcia-Mesa Y, Kan J, Mo X, Khalili K. 2014. RNA-directed gene editing specifically eradicates latent and prevents new HIV-1 infection. Nat Commun 5:3345. https://doi.org/10.1038/ncomms4345.

5. Kaminski R, Bella R, Yin C, Otte J, Ferrante P, Gendelman HE, Li H, Booze R, Gordon J, Hu W, Khalili K. 2016. Excision of HIV-1 DNA by gene editing: a proof-of-concept in vivo study. Gene Ther 23:696. https://doi.org/10.1038/gt.2016.45.

6. Kaminski R, Chen Y, Fischer T, Tedaldi E, Napoli A, Zhang Y, Kan J, Hu W, Khalili K. 2016. Elimination of HIV-1 genomes from human T-lymphoid cells by CRISPR/Cas9 gene editing. Sci Rep 6:22555. https://doi.org/10.1038/srep22555.

7. Kaminski R, Bella R, Yin C, Otte J, Ferrante P, Gendelman HE, Li H, Booze R, Gordon J, Hu W, Khalili K. 2016. Excision of HIV-1 DNA by gene editing: a proof-of-concept in vivo study. Gene Ther 23:690–695. https://doi.org/10.1038/gt.2016.41.

8. Dampier W, Sullivan NT, Chung CH, Mell JC, Nonnemacher MR, Wigdahl B. 2017. Designing broad-spectrum anti-HIV-1 gRNAs to target patient-derived variants. Sci Rep 7:14413. https://doi.org/10.1038/s41598-017-12612-z.

9. Agostini S, Ali H, Vardabasso C, Fittipaldi A, Tasciotti E, Cerese A, Bugatti A, Rusnati M, Lusie M, Giacca M. 2017. Inhibition of non canonical HIV-1 Tat secretion through the cellular Na+K+-ATPase blocks HIV-1 infection. EBioMedicine 21:170–181. https://doi.org/10.1016/j.ebiom.2017.06.011.

10. Ophinni Y, Inoue M, Kotaki T, Kameoka M. 2018. CRISPR/Cas9 system targeting regulatory genes of HIV-1 inhibits viral replication in infected T-cell cultures. Sci Rep 8:7784. https://doi.org/10.1038/s41598-018-26190-1.

11. Yin L, Hu S, Mei S, Sun H, Xu F, Li J, Zhu W, Liu X, Zhao F, Zhang D, Cen S, Liang C, Guo F. 2018. CRISPR/Cas9 inhibits multiple steps of HIV-1 infection. Hum Gene Ther 29:1264–1276. https://doi.org/10.1089/hum.2018.018.

12. Bruner KM, Murray AJ, Pollack RA, Soliman MG, Laskey SB, Capoferri AA, Lai J, Strain MC, Lada SM, Ho H, Richman D, Deeks SG, Siliciano JD, Siliciano RF. 2016. Defective proviruses rapidly accumulate during acute HIV-1 infection. Nat Med 22:1043–1049. https://doi.org/10.1038/nm.4156.

13. Burland TG. 2000. DNASTAR’s Lasergene sequence analysis software. Methods Mol Biol 132:71–91. https://doi.org/10.1385/1-59259-192-2:71.

14. Yuan X, Matsuda Z, Matsuda M, Essex M, Lee TH. 1990. Human immunodeficiency virus vpr gene encodes a virion-associated protein. AIDS Res Hum Retroviruses 6:1265–1271. https://doi.org/10.1089/aid.1990.6.1265.