RT-LAMP assay for detecting lentiviruses

February 4, 2017

1 Introduction

Tests for known lentivirus retroviruses with a high viral diversity such as HIV are very specific and could miss divergent HIV strains [1][2]. In [3], a number of studies designing degenerate PCR primers for detecting lentiviruses are reviewed. In [4], PCR primers targetting a conserved region of the pol gene across five different lentivirus sequences were designed. The goal of the study was to look for evidence of a lentivirus in patients with rhematoid arthritis.

equine infectious anemia virus (EIAV; Genbank no. M16575)
visna-maedi virus (VISNA; Genbank no. M10608)
caprine arthritis-encephalitis virus(CAEV; Genbank no. K03327)
human immunodeficiency virus type 1 (HIV-I; Genbank no. K03455)
HIV-2 (Genbank no. M15390)

All representative sequences were complete genomes with the exception of CAEV, which was only the pol gene.

NCBI Genbank search:
M16575 M10608 K03327 K03455 M15390
https://www.ncbi.nlm.nih.gov/nuccore/M16575,M10608,K03327,K03455,M15390
Download file in FASTA format
multiple sequence alignment with Clustal O

The following degenerate PCR primers were used:
Forward primer 5' to 3':
CAATGGCCMTTVACDGAAGARAAAHTA
Reverse primer 5' to 3':
TARGGGTAKWGWAAARTATGCATCHCC

reverse complement of reverse primer:
GGDGATGCATAYTTTWCWMTACCCYTA

The degeneracy of a sequence is the number of unique sequence combinations it contains, which can be calculated as $d(S) = \prod_{i=1}^{l} |x_i|$.

match
A number of RT-LAMP assays using degenerate primer [5][6]

2 Design

design constraints balance minimal design/development time, minimal equipment requirements, adequate performance.

Primer design tools:
Primer Explorer

Figure 1: Lentivirus Taxonomy

LAVA

[7]

    Primer validation:

eLAMP [8]

    see if simulated analysis matches experimental result from previous study as form of evaluation since eLAMP paper did not verify actual amplification against simulated results.

2.1   Sample Collection

minimal sample volume, blood drop from lanclet

2.2   Lysis

[9]

2.3   Reaction

References

[1] I. Bártolo and N. Taveira, *HIV-1 Diversity and Its Implications in Diagnosis, Transmission, Disease Progression, and Antiretroviral Therapy.* INTECH Open Access Publisher, 2012.

[2] L. M. Luft, M. J. Gill, and D. L. Church, "Hiv-1 viral diversity and its implications for viral load testing: review of current platforms," *International Journal of Infectious Diseases*, vol. 15, no. 10, pp. e661–e670, 2011.

[3] C. Voisset, R. A. Weiss, and D. J. Griffiths, "Human rna rumor viruses: the search for novel human retroviruses in chronic disease," *Microbiology and Molecular Biology Reviews*, vol. 72, no. 1, pp. 157–196, 2008.

[4] F. S. D. Giovine, S. Bailly, J. Bootman, N. Almond, and G. W. Duff, "Absence of lentiviral and human t cell leukemia viral sequences in patients with rheumatoid arthritis," *Arthritis & Rheumatism*, vol. 37, no. 3, pp. 349–358, 1994.

[5] S. Fukuta, T. Iida, Y. Mizukami, A. Ishida, J. Ueda, M. Kanbe, and Y. Ishimoto, "Detection of japanese yam mosaic virus by rt-lamp," *Archives of Virology*, vol. 148, no. 9, pp. 1713–1720, 2003.

[6] M. R. Nunes, J. L. Vianez, K. N. Nunes, S. P. da Silva, C. P. Lima, H. Guzman, L. C. Martins, V. L. Carvalho, R. B. Tesh, and P. F. Vasconcelos, "Analysis of a reverse transcription loop-mediated isothermal amplification (rt-lamp) for yellow fever diagnostic," *Journal of virological methods*, vol. 226, pp. 40–51, 2015.

[7] C. Torres, E. A. Vitalis, B. R. Baker, S. N. Gardner, M. W. Torres, and J. M. Dzenitis, "Lava: an open-source approach to designing lamp (loop-mediated isothermal amplification) dna signatures," *BMC bioinformatics*, vol. 12, no. 1, p. 240, 2011.

[8] N. R. Salinas and D. P. Little, "Electric lamp: Virtual loop-mediated isothermal amplification," *ISRN bioinformatics*, vol. 2012, 2012.

[9] K. A. Curtis, D. L. Rudolph, and S. M. Owen, "Rapid detection of hiv-1 by reverse-transcription, loop-mediated isothermal amplification (rt-lamp)," *Journal of virological methods*, vol. 151, no. 2, pp. 264–270, 2008.