Albany College of Pharmacy AND HEALTH SCIENCES

# Quantification of HIV-1 1-LTR Cycle DNA by Using a Nested Real Time PCR

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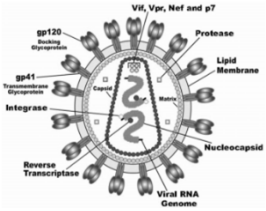
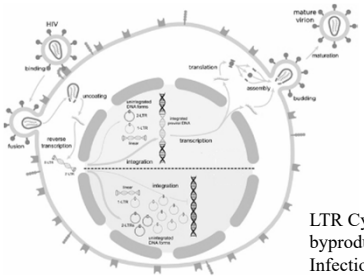
## Introduction

- HIV is classified as a retrovirus.
- Transmitted through bodily fluids such as blood, semen, and breastmilk
- Leads to the death of CD4 T cells, transitioning the disease into " Acquired Immunodeficiency Disease" (AIDS)
- No cure, but can be treated with Antiretroviral Therapy (ART)



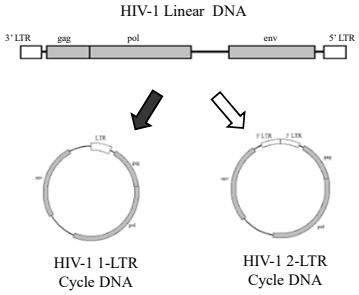

## HIV-1 Life Cycle



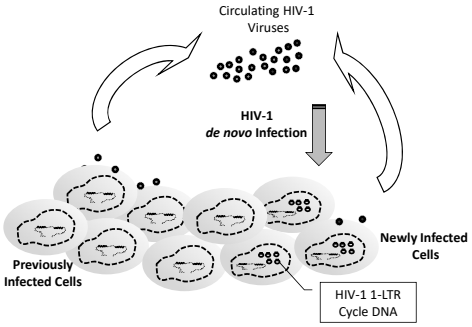

LTR Cycle DNAs are byproducts of HIV-1 Infection

Adapted from Martinez-Picado J et al Retrovirology. 2018; 15: 15.

## HIV-1 Unintegrated Linear DNA can be Circularized into LTR Cycle DNA

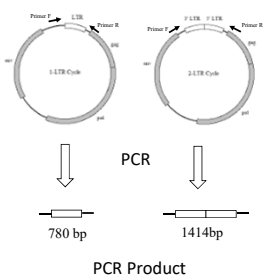



## HIV-1 LTR Cycle DNA is a Biomarker for *de novo* Infection

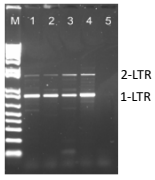


Methods and Results

## PCR Amplification HIV-1 1-LTR Cycle and 2-LTR Cycle DNA from HIV-1 Infected Cells.

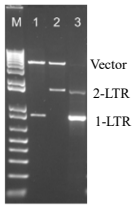



PCR Product




DNA from HIV-1 infected:
1. MT4 cells.
2. THP1 cells.
3. HCT116 $p53^{+/+}$ cells.
4. HCT116 $p53^{-/-}$ cells.
5. Negative.

## Cloning of HIV-1 1-LTR Cycle and 2-LTR Cycle PCR Products


1. HIV-1 1-LTR Clone digested by EcoR I.
2. HIV-1 2-LTR Clone digested by EcoR I.
3. HIV-1 LTR PCR products

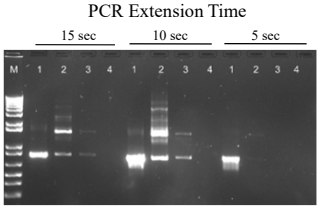
## Optimizing PCR to amplify HIV-1 1-LTR Cycle DNA Exclusively

PCR Product

2-LTR Cycle
1414bp

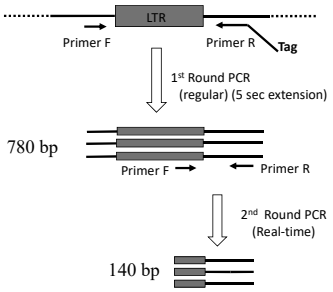
1-LTR Cycle
780 bp

PCR Extension Time

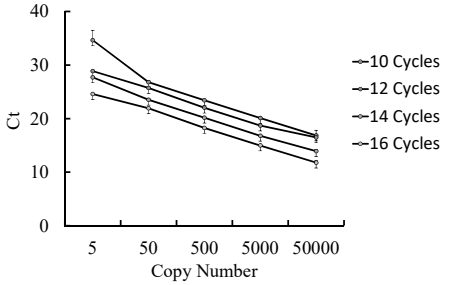



1. 1-LTR Clone.
2. 2. 2-LTR Clone.
3. Full genome HIV-1 clone pNL-43.
4. Negative

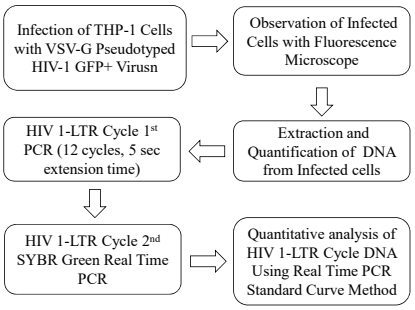
## Strategy to Detect HIV-1 1-LTR Cycle DNA by Using a Nested Real-Time PCR



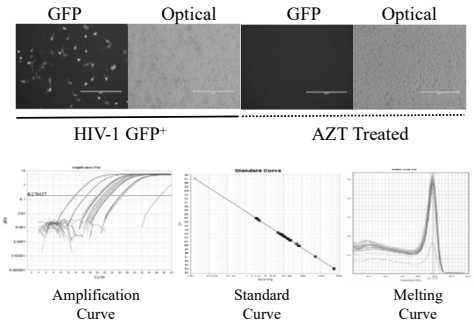

## Impact of 1st PCR Cycle Number to the Linearity of Real Time PCR Standard Curve



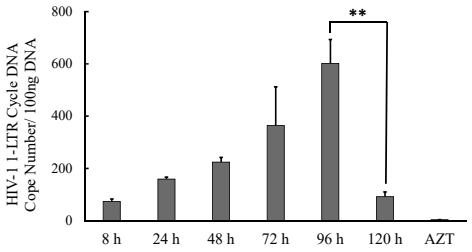
## Experimental procedures to quantify HIV-1 1-LTR DNA in human monocytic cell line THP-1




## Quantitative Detection of HIV-1 1-LTR Cycle DNA by Using a Nested Real-Time PCR




## Quantitative Detection of HIV-1 1-LTR Cycle DNA by Using a Nested Real-Time PCR




** indicates $p < 0.01$ in a Student's t-test.

## Conclusions and Discussions

We have developed a PCR method that can exclusively amplify 1-LTR DNA with increased sensitivity and specificity.

1) Optimized the extension time in 1st PCR to allow synthesis HIV-1 1-LTR cycle DNA exclusively.
2) The use of a tagged primer ensures that only the 1st PCR products are used as templates in 2nd PCR.
3) Short amplicon size in 2nd PCR significantly increased the efficiency of amplification in real time PCR.
4) The high processivity of the Phusion DNA polymerases avoids generation of short incomplete PCR products which leads to the generation of artificial PCR products.

- HIV-1 1-LTR cycle DNA is a type of episomal DNA that decreases in abundancy over time due to:
  1) Lack of a replication system.
  2) Frequent attacks by cellular nucleases.
- The quantification of HIV-1 1-LTR cycle DNA by real time PCR has the potential to be used as a marker for detecting ongoing *de novo* infection in HIV-1 infected patients.
- The development of a marker capable of detecting ongoing HIV-1 replication is an important step in understanding viral dynamics and in developing therapeutic strategies.

Thank you.

Questions?