# Assay To Measure The Latent Reservoir Of Replication-Competent HIV-1 In Suppressed Patients Based On Ultra Deep Sequencing

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## Abstract

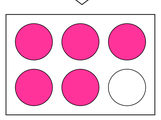
**Background:** Viral outgrowth assay (VOA) is a widely used culture assay to measure the latent HIV-1 reservoir harboring replication-competent HIV-1 in resting CD4+ T cells in patients on HAART. However, the assay is costly, and both labor and resource intensive. To overcome some of these issues with the VOA, we designed an assay using ultra deep sequencing (UDS), which directly analyzes the number of different sequences of the induced viruses to score the number of latently HIV-infected resting CD4+ T cells. In this study, we tested the premise whether the viral sequences derived from two different proviruses are genetically distinct, since the assay involves a bulk culture.
**Methods:** To analyze viruses derived from different VOA culture wells scored as p24 positive, the viral samples derived from different culture wells were assigned with a specific Barcode and subjected to sequence analysis of the V1-V3 region of *env* sequences using the Primer ID-based paired-end MiSeq platform. A total of nine patient samples, two acute and seven chronic, were analyzed by UDS. Phylogenetic trees were generated by using consensus sequences created from sequences with the identical Primer ID and were used to detect distinct viral lineages present in the individual culture supernatant. For chronic patient samples, IUPM values were determined by using distinct viral lineages detected and the adjusted number of patient-derived resting CD4+ T cells used for VOA.
**Results:** Approximately ≅50% of the viral lineages derived from each chronic patient were distinct. In contrast, all viral lineages derived from each acute patient were homogeneous. When IUPM values determined by UDS analysis were compared to the IUPM values obtained from VOA, we observed approximately 2-fold higher IUPM values than the IUPM values determined by VOA. We also observed a significant positive correlation between the number of viral lineages observed per well and the number of resting T cells present per well.
**Conclusions:** The results suggest that ≅50% of the viral lineages induced from different cells derived from chronic patients were distinct. Thus, the UDS assay is applicable for samples derived from chronic patients. The multiplexing ability of the assay improves the efficiency for the throughput capacity.

## Primer ID-Based Deep Sequencing Assay Combined With Bulk Culture To Measure Latent HIV-1 Reservoir

- Directly count the number of distinct sequences of the viruses induced to replicate in cultured resting T cells by using deep sequencing analysis.
- Primer ID significantly reduces errors introduced at PCR or sequencing steps.
- Bulk-culture assay could improve work flow with the current gold-standard assay by reducing time, labor, and cost.

## Primer ID-Based Deep Sequencing Assay and IUPM calculation Scheme

2 million resting T cells in 5 wells and one control well without resting T cells

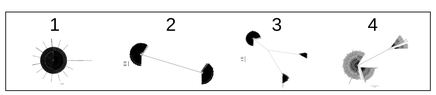


*5 replicas (10 million resting T cells total)*

*Culture for 7-14 days*

Ultra deep sequencing analysis of individual culture supernatant

Count distinct viral lineages in each culture supernatant

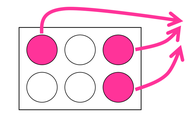



- Generate phylogenetic tree using all distinct viral lineages
- Count the number of wells containing the same viral lineage
- Determine the titer of individual viral lineage based on Poisson distribution
- Add all the titers from individual viral lineage

IUPM (Infection Unit Per Million)

## Hypothesis

***Viral sequences induced from two different cells are distinct***

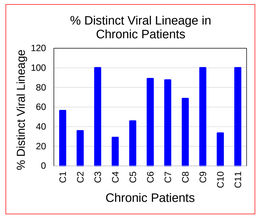


**Cultures from Viral Outgrowth Assay (VOA)**

**Viruses scored as p24 positive for the presence of HIV-1 from viral outgrowth assay**

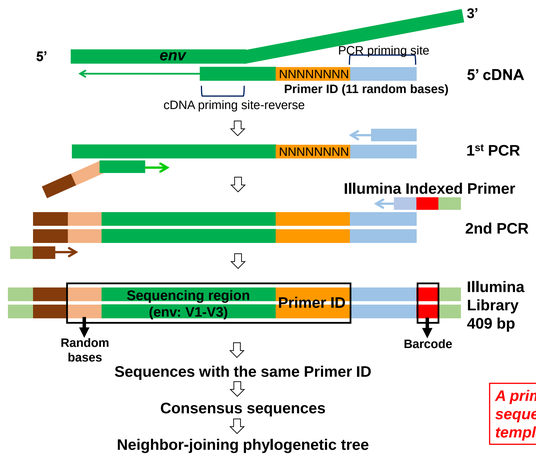
**Ultra deep sequencing analysis**

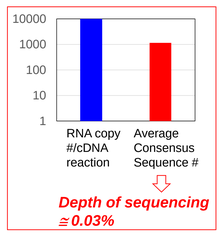
**Phylogenetic tree to compare viral lineages derived from different wells**




*Distinct viral sequences are observed when cells are derived from chronic patients.*

## MiSeq (300 bp paired-end sequencing) Library of cDNAs Derived From Viral RNA

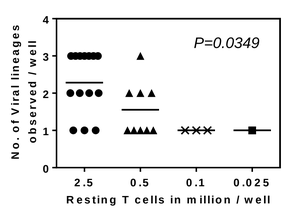


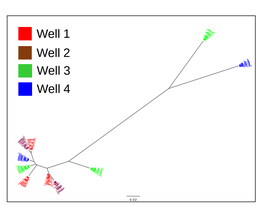

***Depth of sequencing ≅0.03%***

***A primer ID (individual consensus sequence) represents each template used for cDNA reaction.***

## Viral Lineage (s) Derived From Individual Culture Well In Outgrowth Assay



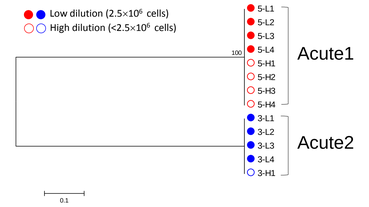

*Positive correlation between the number of viral lineages observed and the number of resting T cells per individual well*




*Viral lineages derived from a chronic patient. Four different culture wells were randomly chosen. Viral lineages derived from each well are color-coded.*

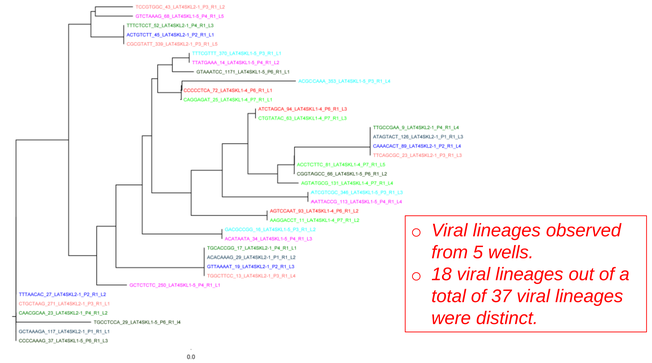
## Most Viral Sequences *(env)* Induced From Different Cells Derived From Chronic Patients Are Distinct

### ➢ Viral Sequences Derived From Acute Patients




*Viral sequences induced from different cells derived from acute patients are homogeneous.*

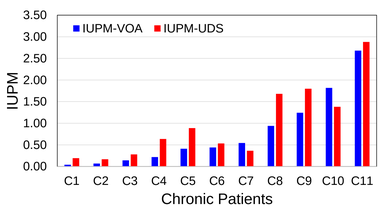
### ➢ Viral Sequences Derived From Chronic patients

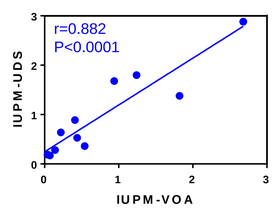



- *Viral lineages observed from 5 wells.*
- *18 viral lineages out of a total of 37 viral lineages were distinct.*

▪ *Each well is color-coded*

### ➢ IUPM-Ultra Deep Sequencing (UDS) compared to IUPM-VOA




## Summary

- We were able to directly analyze the number of different sequences of the viruses induced to grow in viral outgrowth assay using ultra deep sequencing (UDS) to score the number of latently HIV-1 infected resting CD4+ T cells.
- Distinct Viral sequences were observed when viruses were induced from cells derived from chronic patients, however, viral sequences induced from different cells derived from acute patients were homogeneous suggesting that this assay is not ideal for samples derived from acute patients
- Positive correlation was observed between the number of viruses observed and the number of resting T cells per well.
- Infectious Unit Per Million (IUPM) values determined by UDS method were comparable to those determined by VOA.
- A strong correlation between IUPM-VOA and IUPM-UDS validates Primer-ID based UDS culture assay to measure the latent reservoir of replication-competent HIV-1 in resting CD4+ T cells in suppressed patients.