

An Analysis of Genital Tract Derived HIV from Heterosexual Transmission Pairs



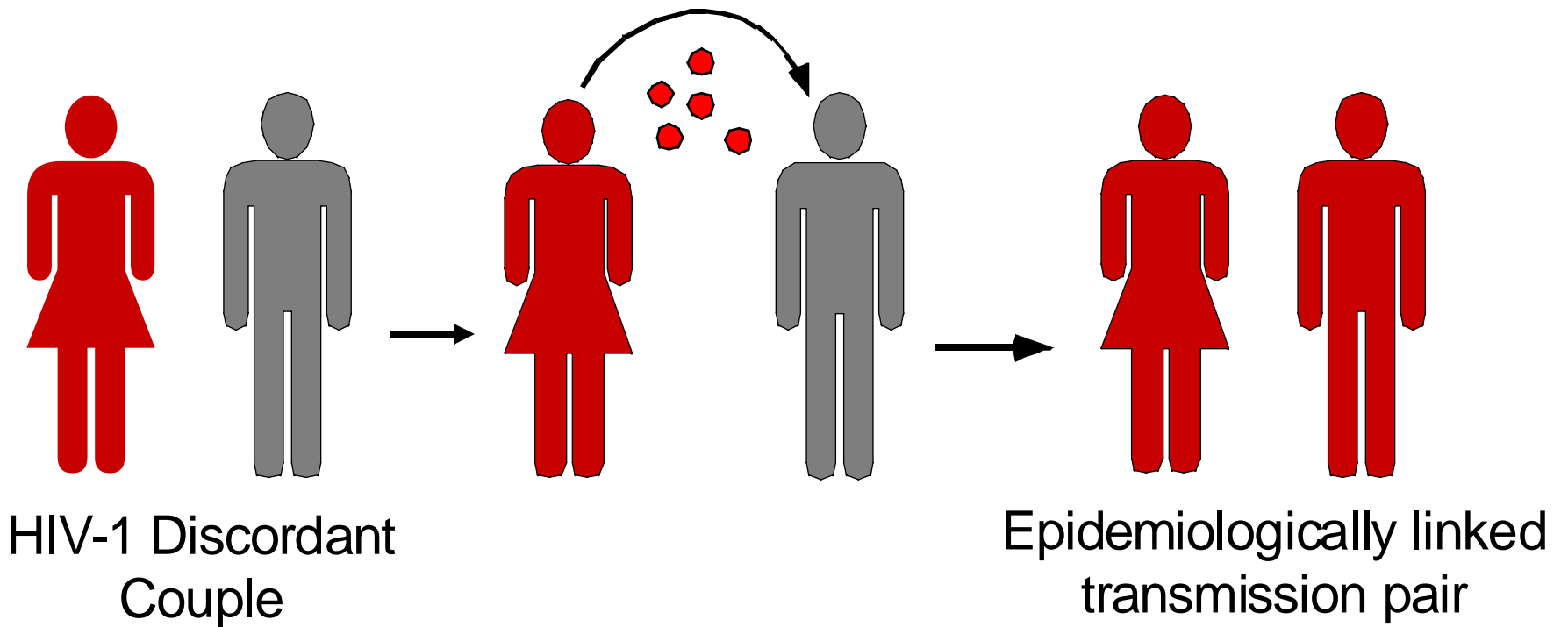
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Background

- A majority of HIV-1 infections occur through heterosexual exposure to seminal fluid and vaginal secretions of infected individuals.
- We have studied heterosexual transmission in 2 large HIV-1 discordant couple cohorts (S. Allen), recruited in Lusaka, Zambia and Kigali, Rwanda.
 - Despite counseling and condom provision a low frequency of transmission is observed
 - At the time transmission is detected, samples (blood and genital fluids) are collected from the chronically infected (donor) and the newly infected (recipient) partner.

HIV -1 Transmission in Discordant couples

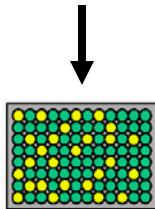


Materials and Methods

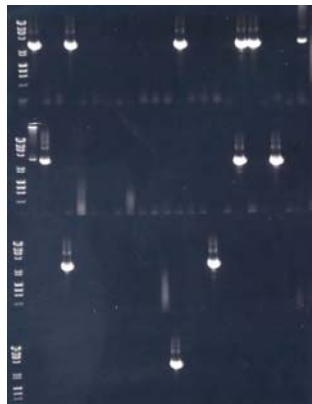
- blood (PBMC and plasma) and genital fluids
- vaginal swabs (Dacron) and semen from chronically infected donors
- collected at 'Day of Seroconversion'
- Single Genome Amplification
 - nested PCR of *env* gene region
- PCR product sequenced in the V1-V4 region
- nucleotide and amino acid alignments of V1-V4 were performed
- phylogenetic and pair wise distance analyses

Single Genome Amplification (SGA) to avoid genome resampling and *in vitro* PCR based recombination

Dilute to
<1 proviral DNA molecule/rx
~90 replicates

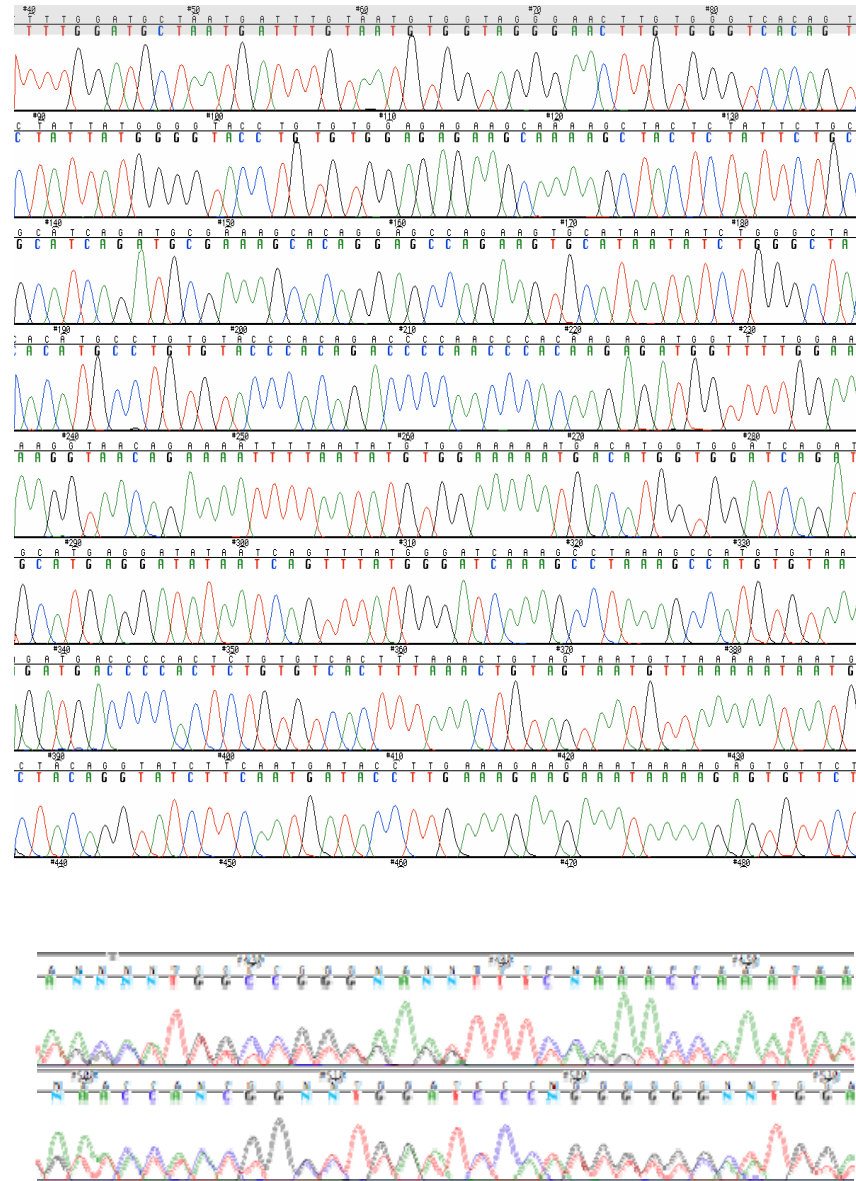


Nested PCR, full-length *env*



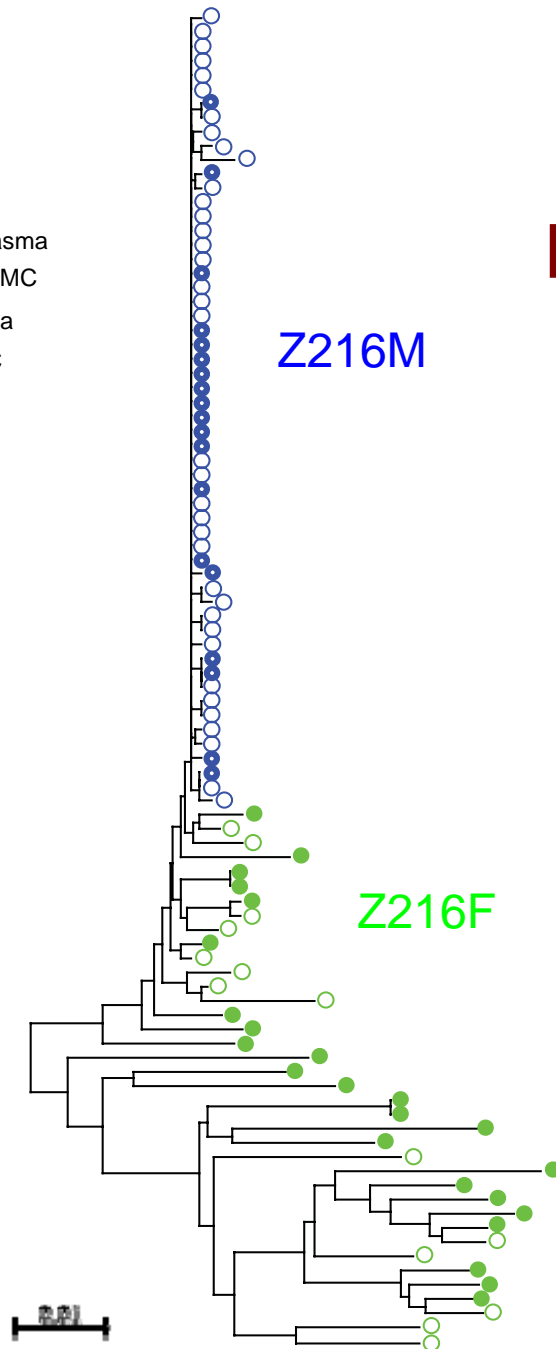
<30% PCR+ wells

SGA
Direct sequencing
multiple genomes



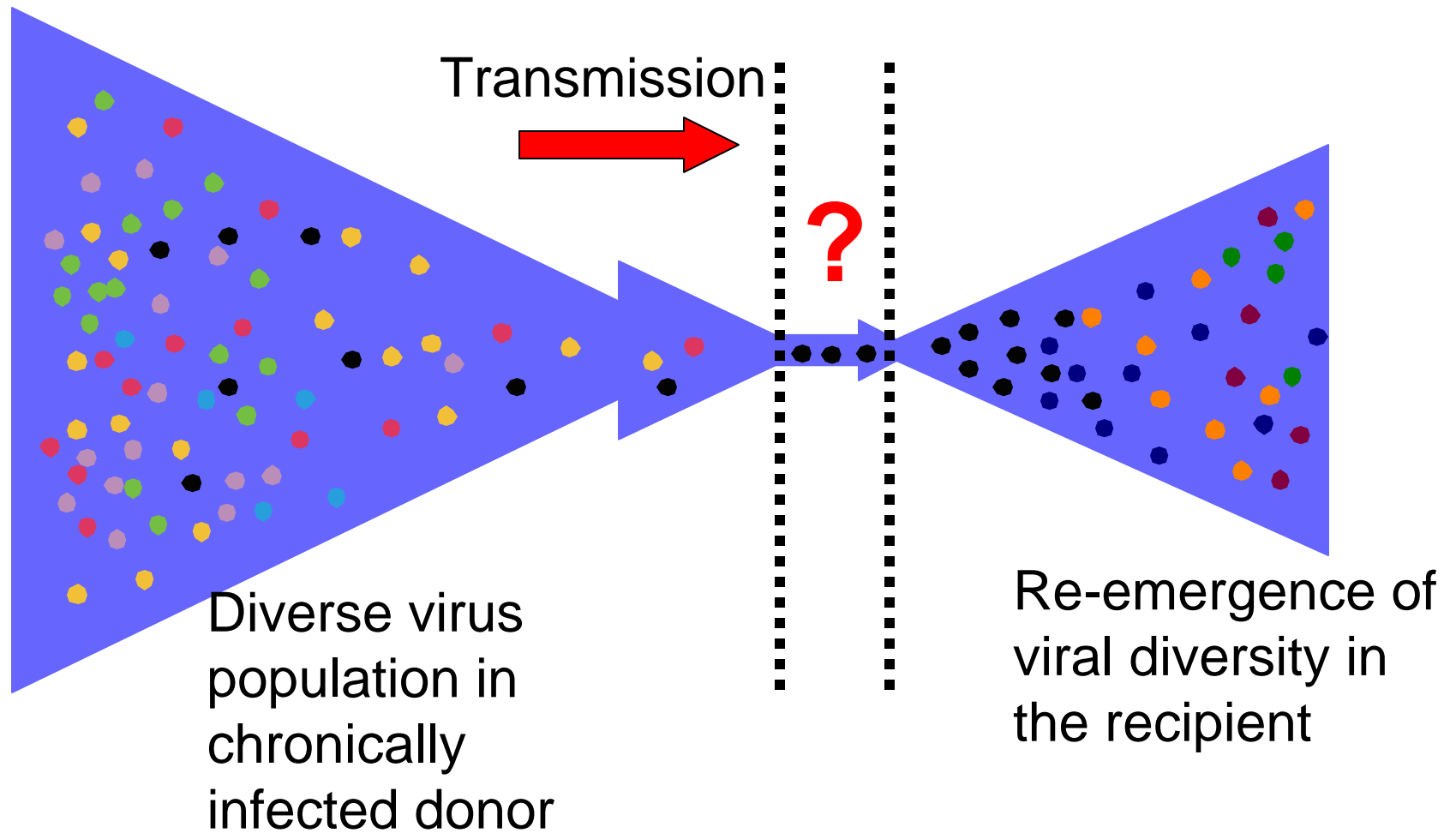
Phylogenetic Analysis of HIV-1 in the Blood reveals a Genetic Bottleneck during Transmission

- Recipient Plasma
- Recipient PBMC
- Donor Plasma
- Donor PBMC



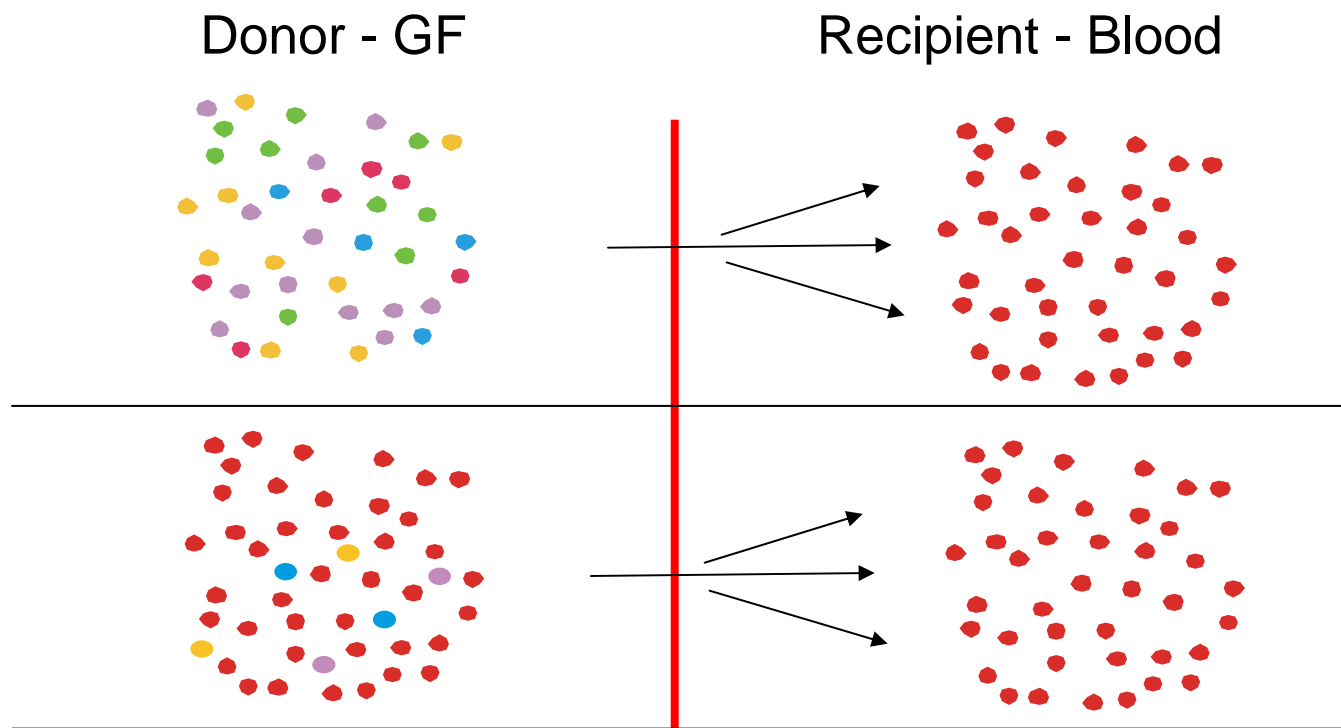
- Blood (PBMC and plasma) from a Zambian epidemiologically-linked transmission pair
- Heterogenous donor quasispecies
- Homogenous recipient quasispecies
- Recipient virus emanates from a single branch of the donor phylogenetic tree

Genetic bottleneck during transmission of HIV



Specific Aim

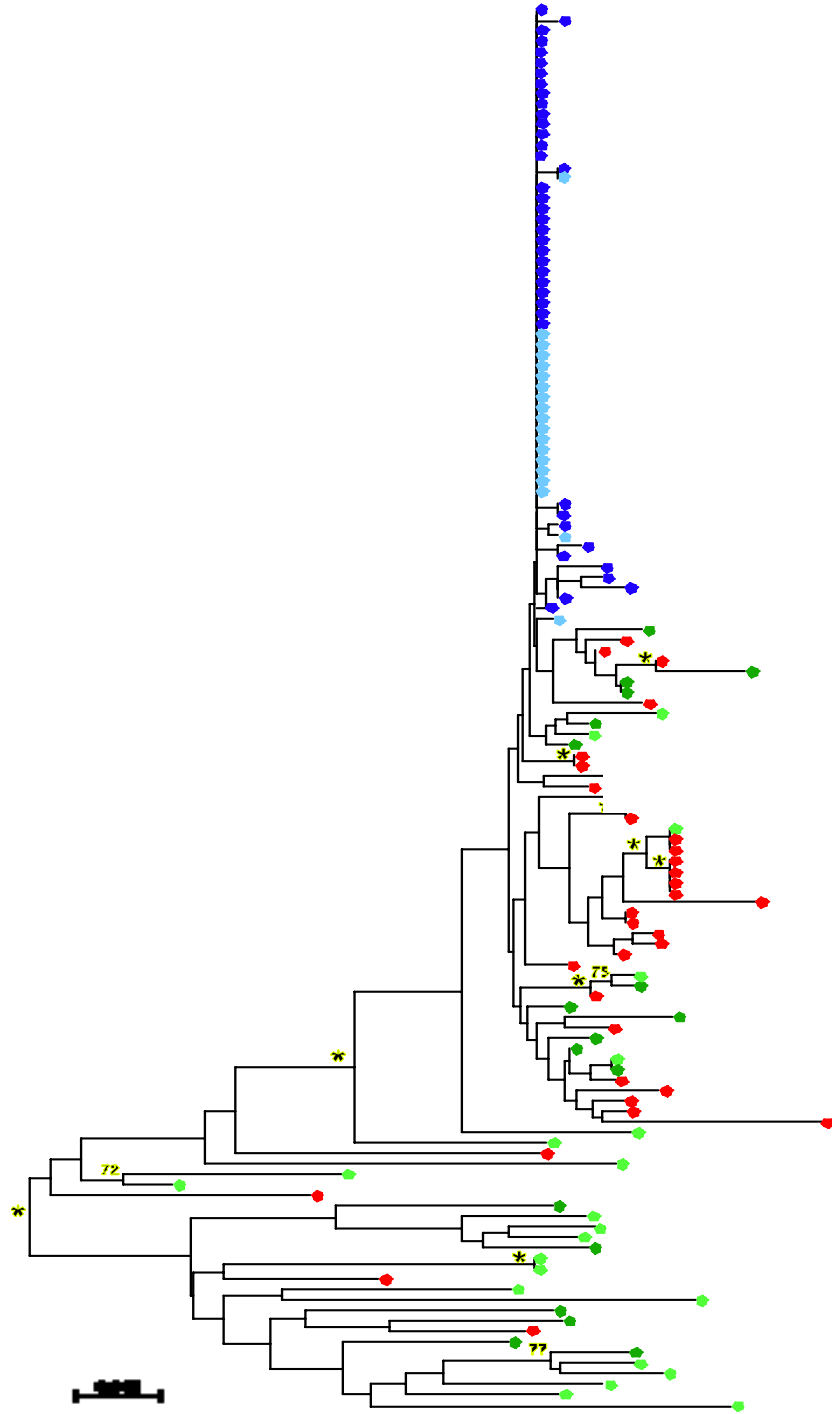
Determine whether the viruses present in donor genital fluids at the time of acute infection are enriched for those that establish infection in the recipient.



Specific Aim

- a) Is the viral population in the genital tract heterogeneous or homogenous?
- b) Is there compartmentalization of the viral population in the genital tract versus the population observed in the blood?
- c) Does the transmitted viral variant reflect the predominant virus population present in the genital fluids of the donor partner?

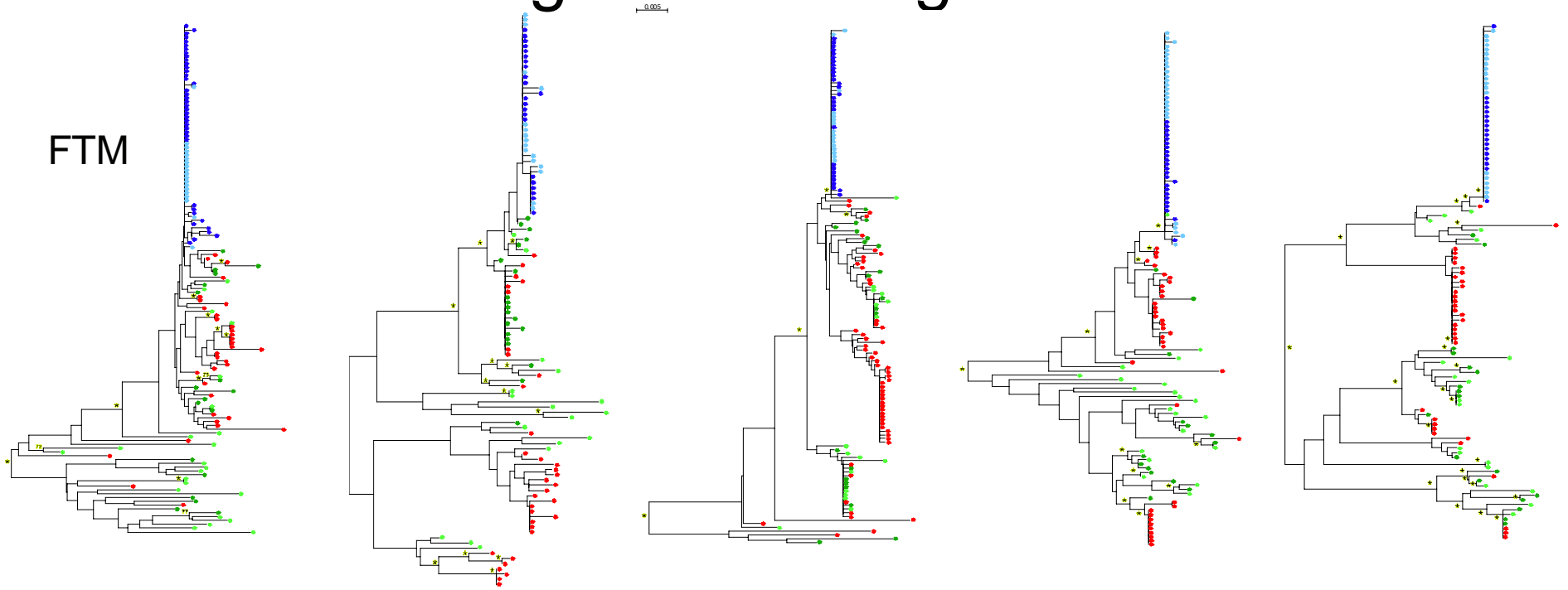
Neighbor Joining Tree Female to Male (FTM) Transmission Pair Z216



Recipient Blood
Donor Blood
Donor Genital Fluids

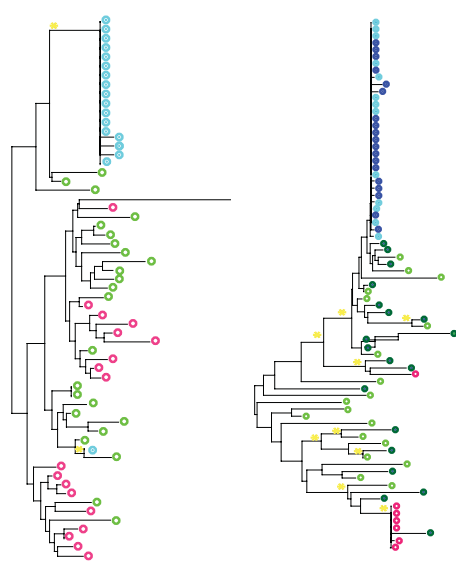
Neighbor Joining Trees

FTM



Recipient Blood
Donor Blood
Donor Genital Fluids

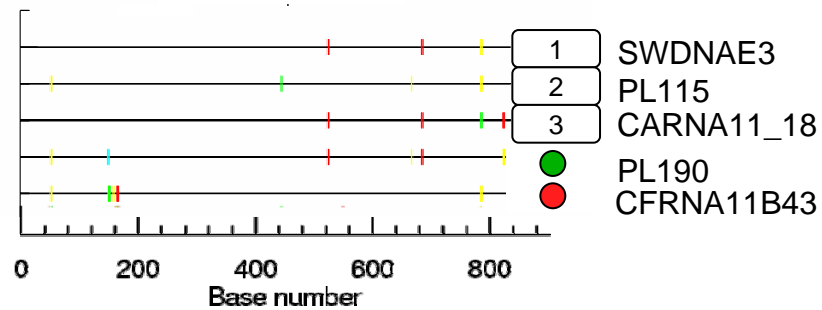
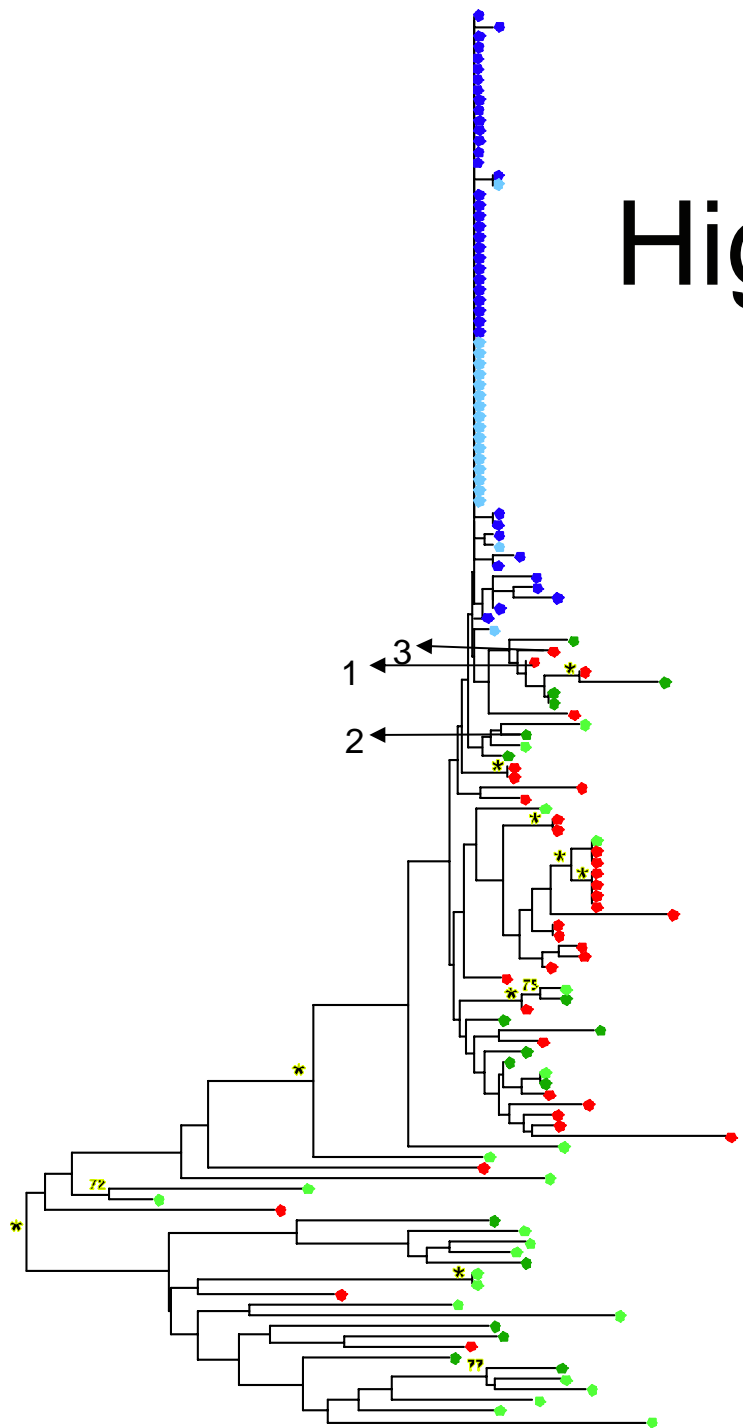
MTF



- heterogeneous virus population in the donor (both blood and genital tract)
- Distinct viral population within the donor genital tract compared to the viral population in the blood

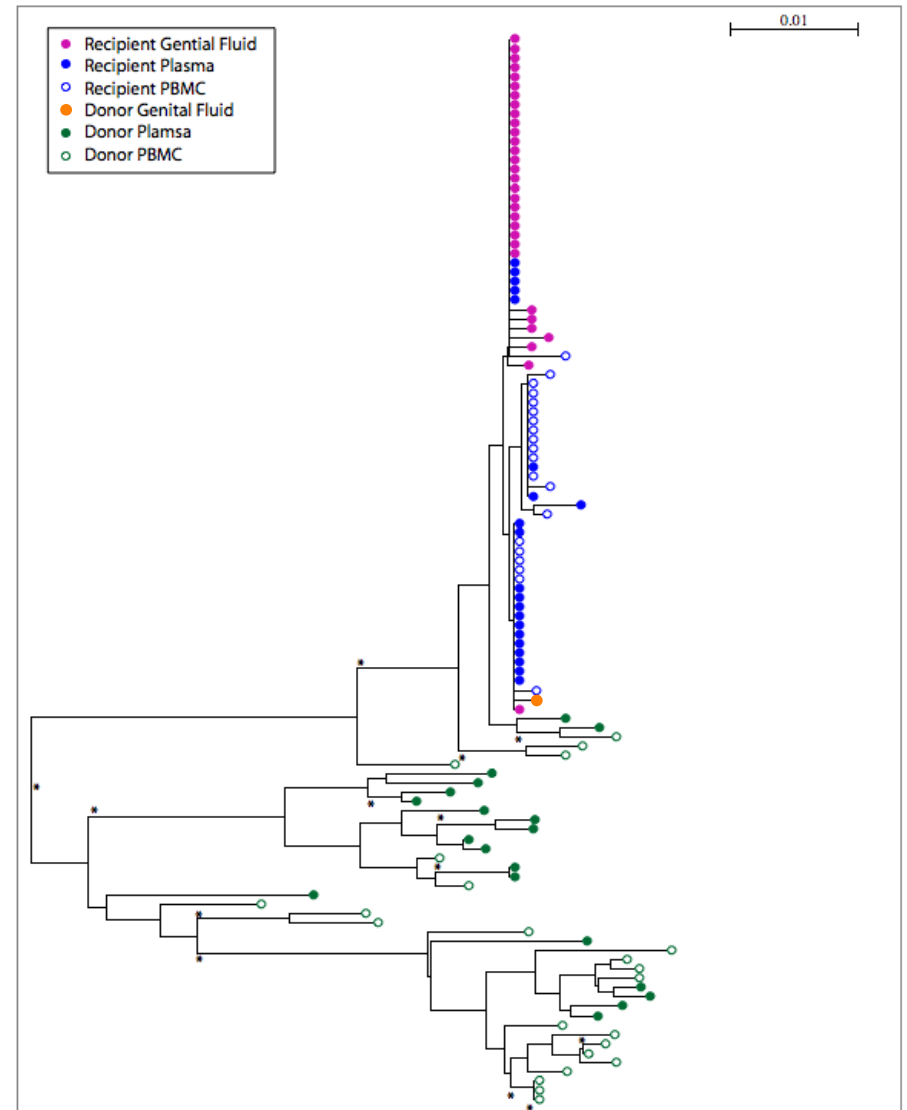
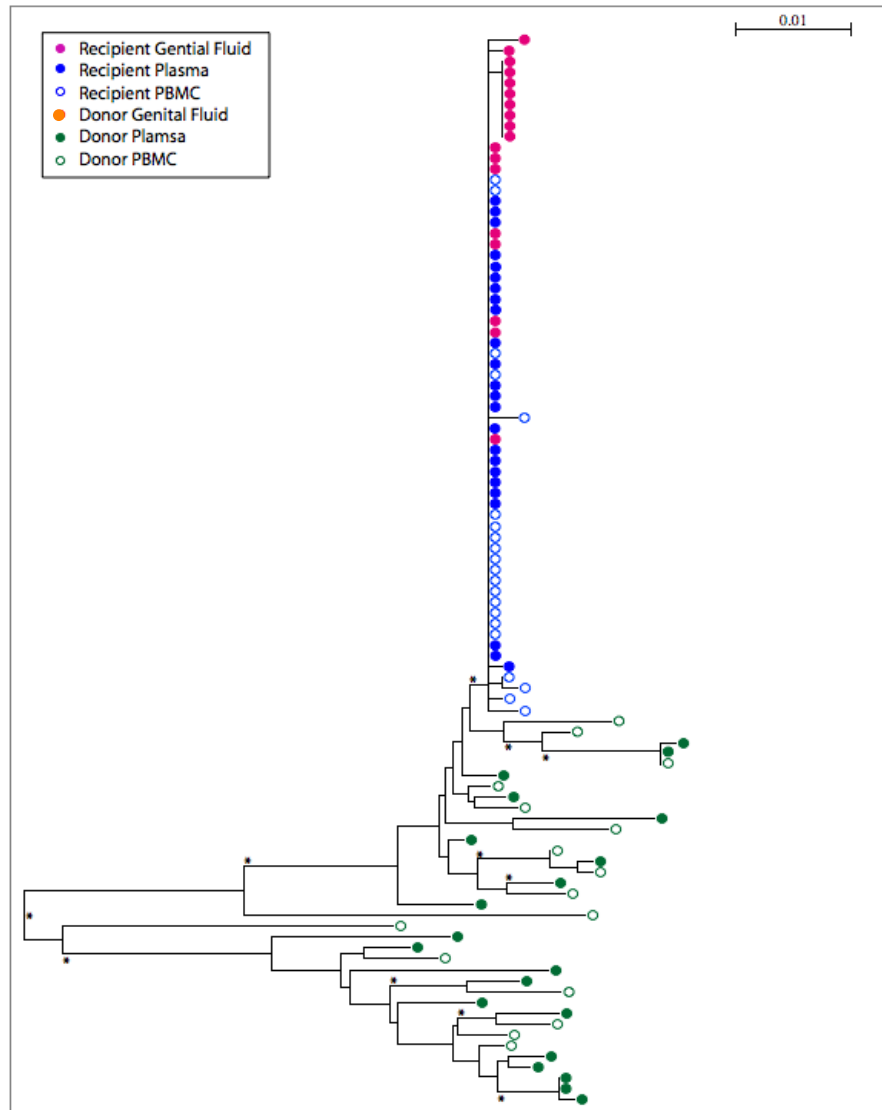
0.005

Highlighter Analysis



- no evidence that the genetic bottleneck observed in the blood between donor and recipient is the result of enrichment for the transmitted viral variant in the donor genital tract
- evidence that a minor variant may be the origin of the transmitted virus

Recipient Neighbor Joining Trees



Summary

- In the majority of epidemiologically-linked transmission pairs an extreme genetic bottleneck is observed during heterosexual transmission
- Some compartmentalization of viral variants within the donor genital tract is observed
- No evidence that the genetic bottleneck is the result of enrichment for the transmitted variant in the donor genital compartment
- Appears that a minor variant is the origin of the transmitted virus



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