High Throughput Single Molecule Sequencing to Characterize Ab-Resistant HIV/SHIV

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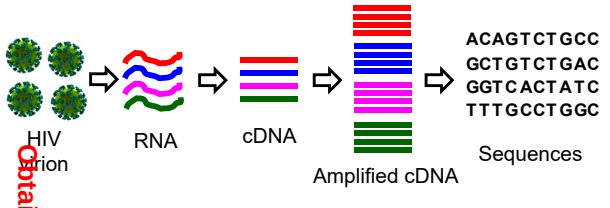
BACKGROUND

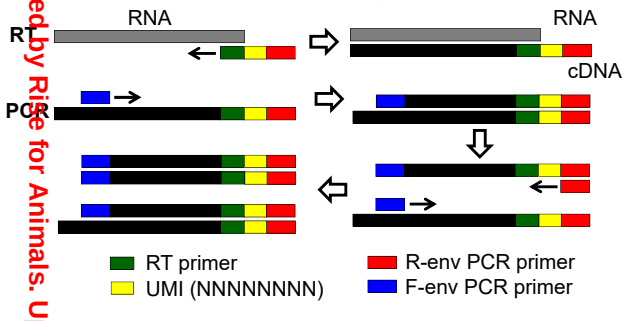
- HIV-specific broadly-neutralizing antibodies (bNAbs) can suppress viremia *in vivo*, but clinical use of bNAbs is limited due to neutralization-resistant viruses. Understanding virus dynamics under bNAb treatment can inform development of this therapeutic approach.
- Conventional single genome (molecule) sequencing (SGS) has low throughput, leading to low sensitivity for minor virus variants.
- High throughput SGS with high accuracy is required to track minor virus variants that may be clinically important. This novel assay may not only help characterize virus dynamics in bNAbs research, but also allow a better understanding of HIV evolution in response to humoral immune pressure.

METHODS

Single molecule sequencing



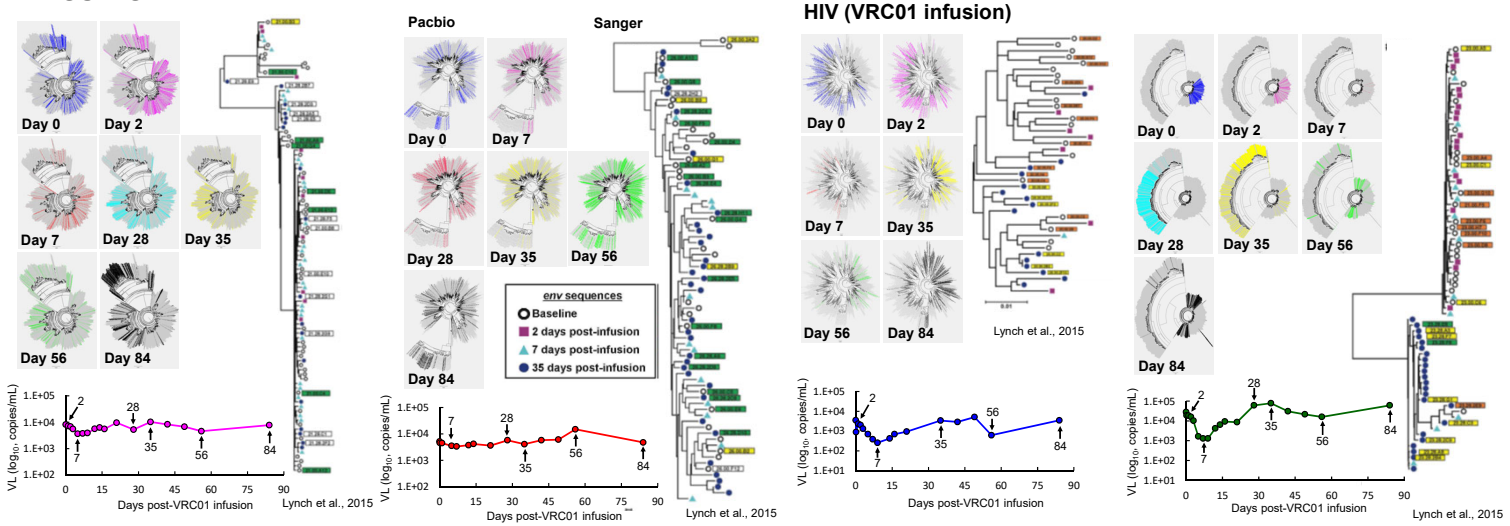
Reverse transcription and full-length *env* PCR



Sequencing (Pacbio) and bioinformatic analysis

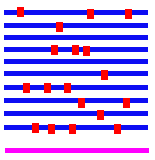
1. Begin with high-quality CCS reads
2. Reorient all sequences (Usearch –orient)
3. Remove primer sequence and filter by length (Cutadapt)
4. Identify the UMI sequence and Bin by UMI (Custom code)
5. Generate consensus sequence (99.9% identity, Custom code)
6. Align and remove frameshifts (Mafft)
7. Perform phylogenetic analysis (FastTree)

RESULTS



- Clear genetic change in virus population over time, without return to pre-infusion pattern → less clear in conventional SGS data than in Pacbio data.
- *env* sequences change after VRC01 infusion in all participants (Day 0 vs Day 84) → most pronounced changes occur after ~ 1 month.

Error quantification



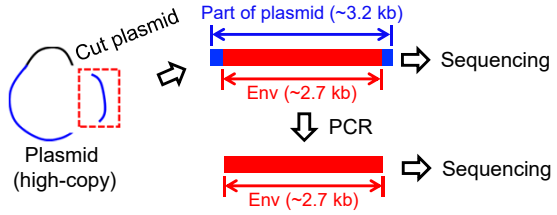
$$= \frac{Reads\ with \geq 1\ mismatch}{Total\ reads} = \frac{8}{10} = 0.8 = 80\%$$

Read number : 10

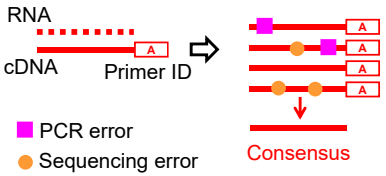
DNA Mismatch (1 base)

Reference sequence

Mismatch rate
87.7 %

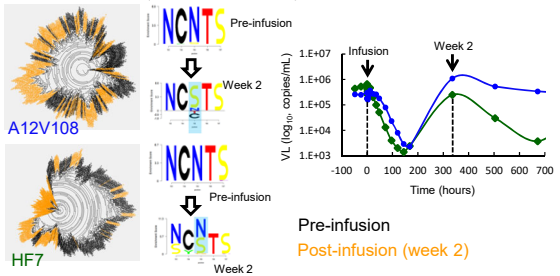


Step	Mismatch rate
All steps	88 %
Sequencing	48 %
env PCR	30 %
IVT + RT	10 %



Error correction analysis	Mismatch rate
Consensus formation analysis (without UMI)	46.55 %
Consensus formation analysis (with UMI)	24.13 %

SHIV (VRC07-523LS infusion)



CONCLUSIONS

- Developed UMI-based Pacbio sequencing method
  - quantification of error rate in each sequencing step
  - great improvement of sequencing accuracy and throughput
- Applied the Pacbio sequencing to SHIV/HIV bNAb study
  - greater detail about virus genetic change after bNAb infusion than conventional SGS
  - a robust method to study dynamics of Ab-resistant HIV