Genetic Drift and Selection in a Captive HSV Population

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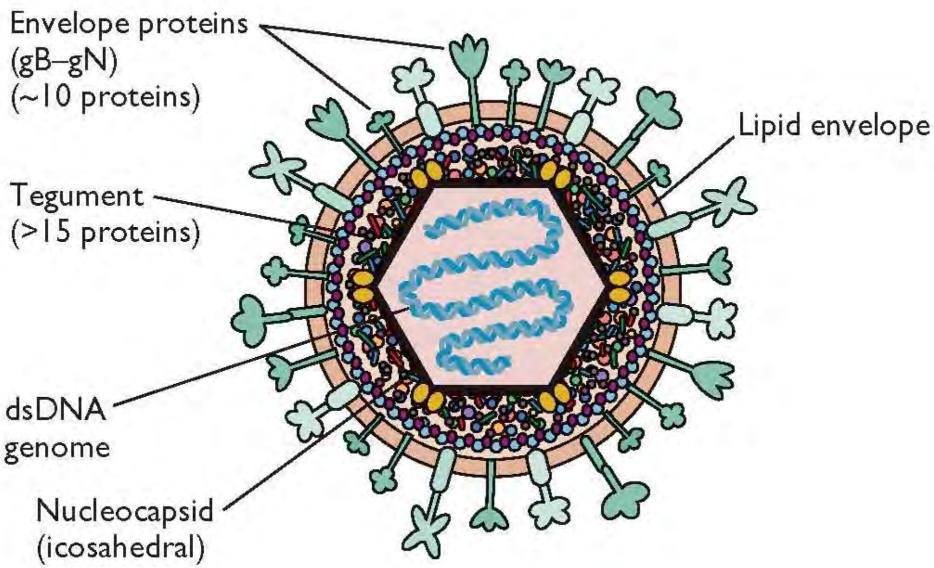
THE HUCK INSTITUTES
OF THE LIFE SCIENCES

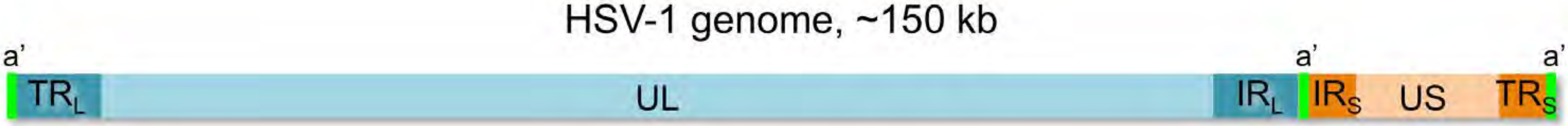
CIDD CENTER FOR INFECTIOUS DISEASE DYNAMICS

PennState
Eberly College
of Science

HSV Background

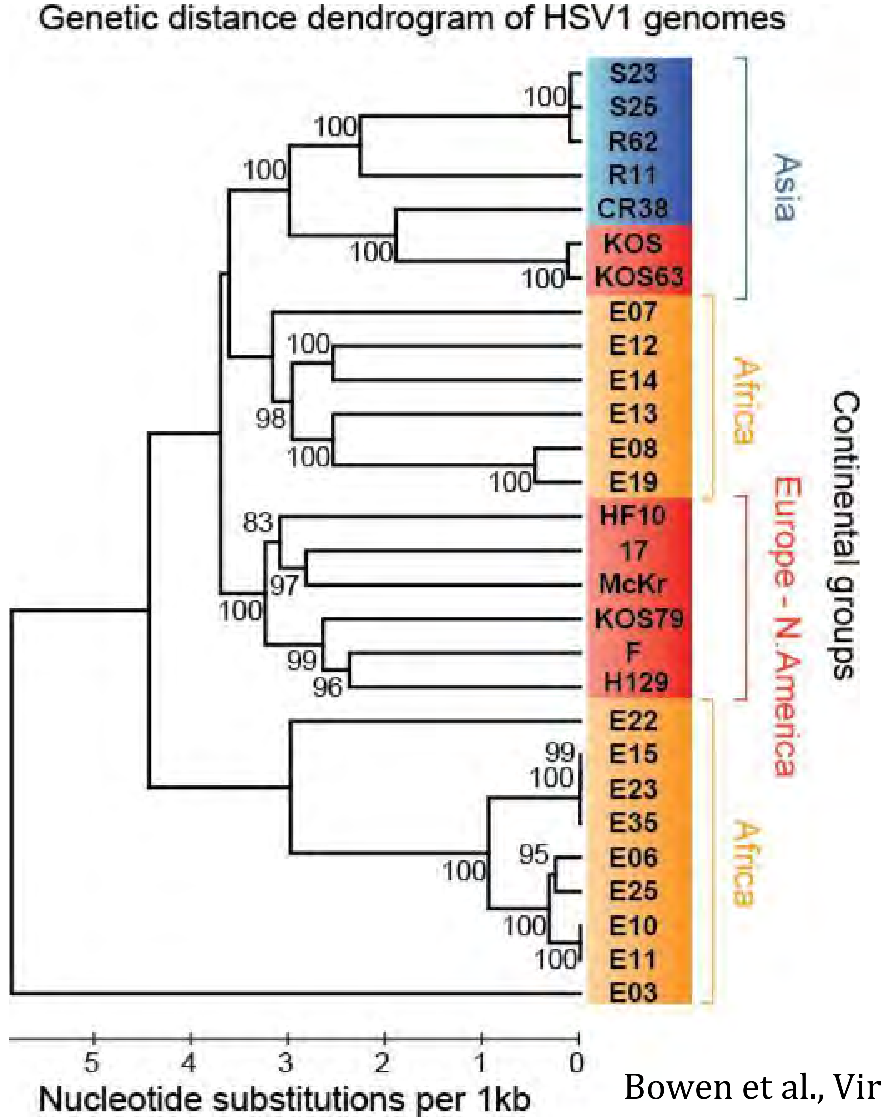


- Extremely prevalent worldwide
- Wide range of disease severity
- α-herpesvirus
- Large dsDNA genome



From Principles of Virology, by S. J. Flint, L. W. Enquist, V. R. Racaniello, A. M. Skalka and Szpara et al., PLoS Pathogens 7(10): e1002282

Genetic Diversity in HSV-1



Bowen et al., Virology, 2016

- HSV-1 genomes are typically similar. (~3-4% variation between strains)
- Similar does not mean identical. (Dozens of AA changes)
- Genomic variation affects how the virus can escape selective pressures. (immune response, antiviral drugs, etc.)

HSV Genetic Diversity Affects Observed Phenotypes

- HSV is well known to evolve in response to selective pressure.

Research Questions

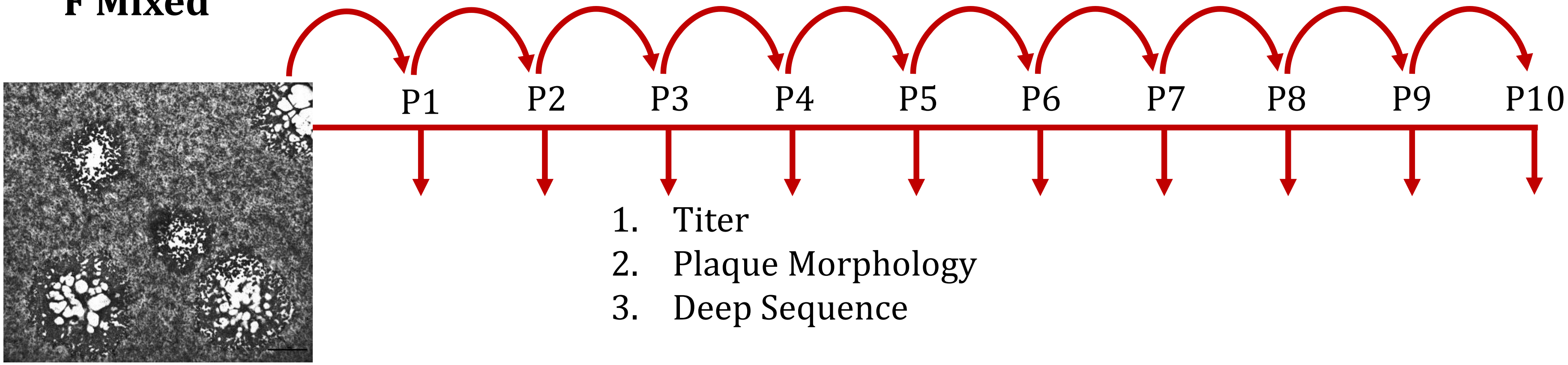
1. How readily does HSV evolve?
    - How fast do genetic variants arise?
    - Mechanism for genetic diversity?
    - Bottlenecking effects?

2. Where/when does genetic drift occur?

Sequential Passage of HSV-1 Populations

HSV-1
F Mixed

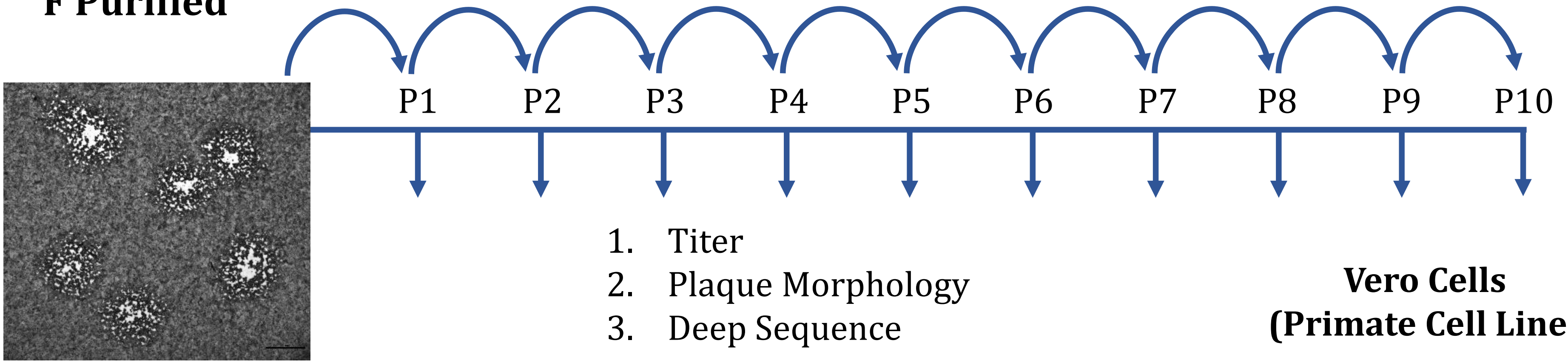
MOI 0.01
Passage 1



1. Titer
2. Plaque Morphology
3. Deep Sequence

HSV-1
F Purified

MOI 0.01
Passage 1



1. Titer
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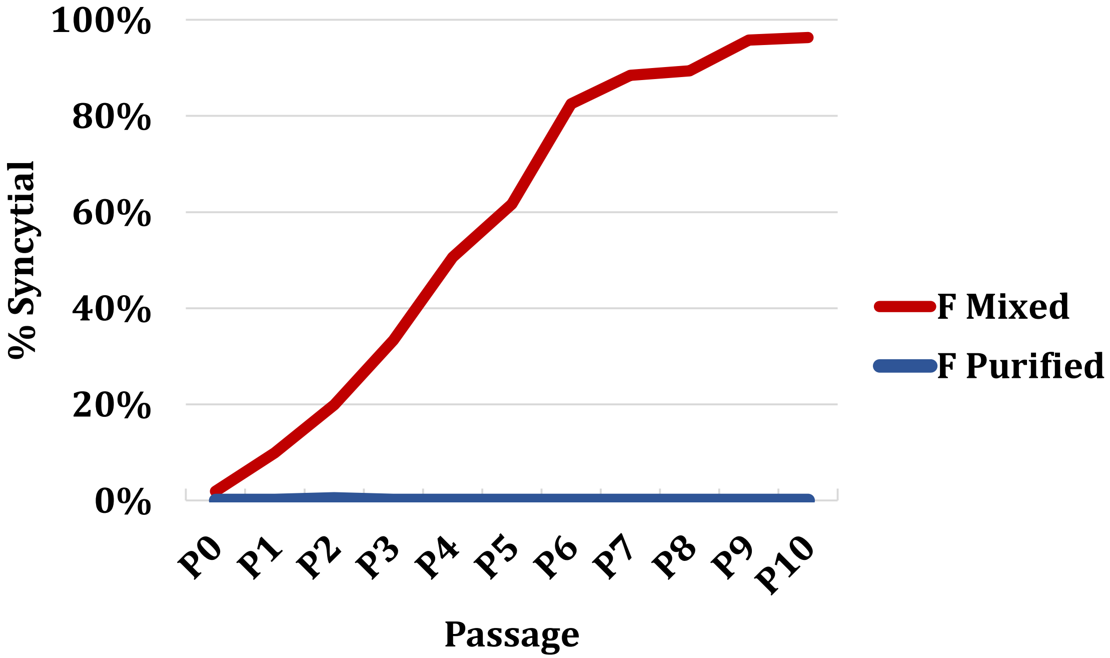
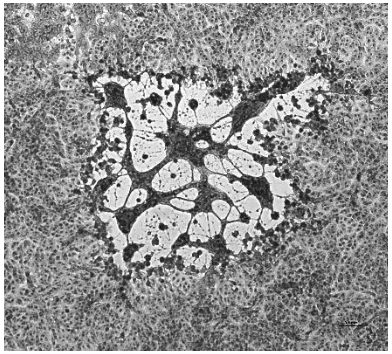
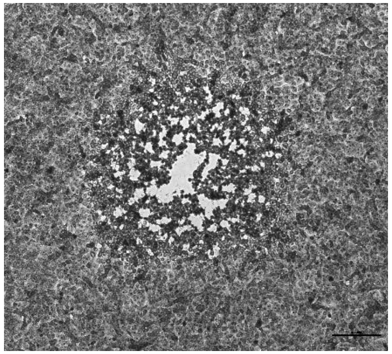
Vero Cells
(Primate Cell Line)

F Mixed Syncytial Population Increases over Passage





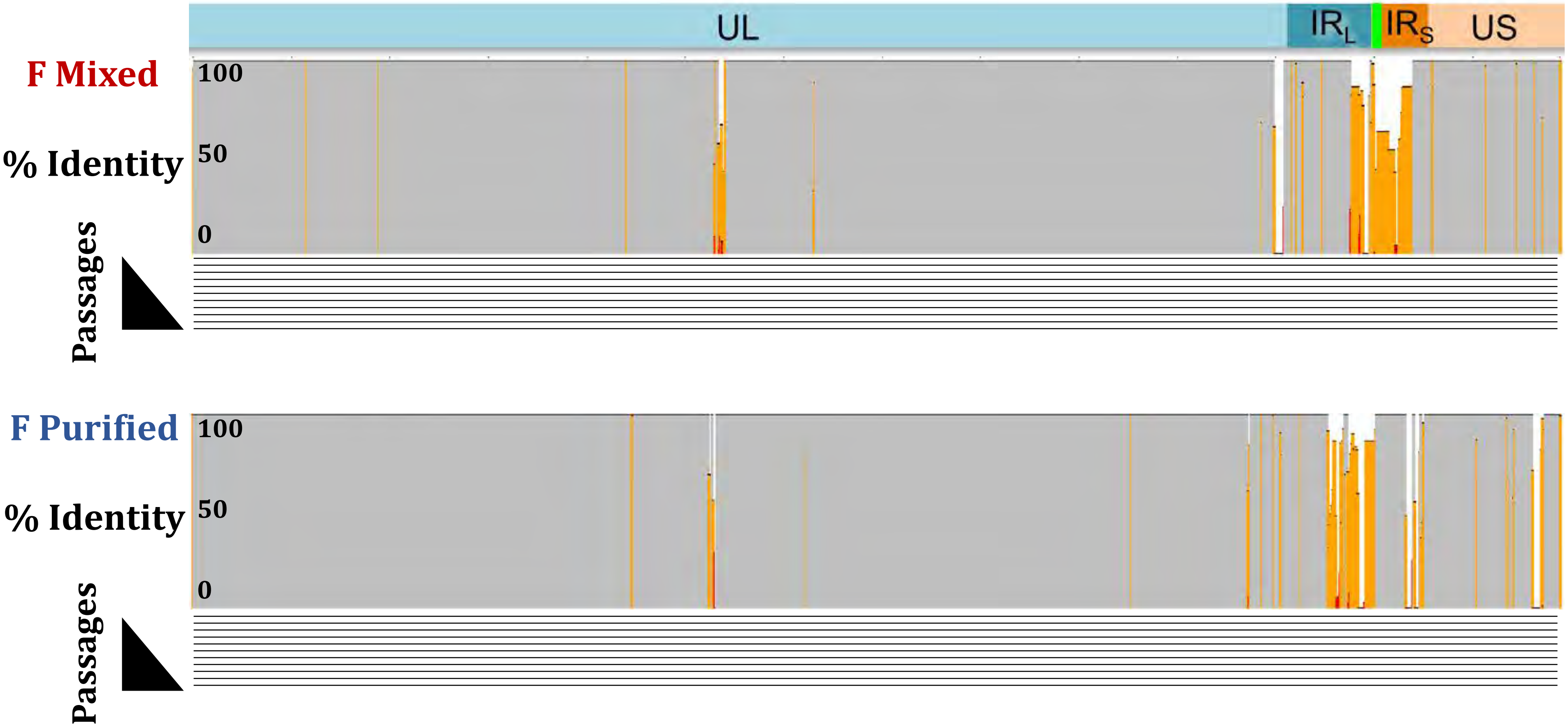
Syncytium



CPE

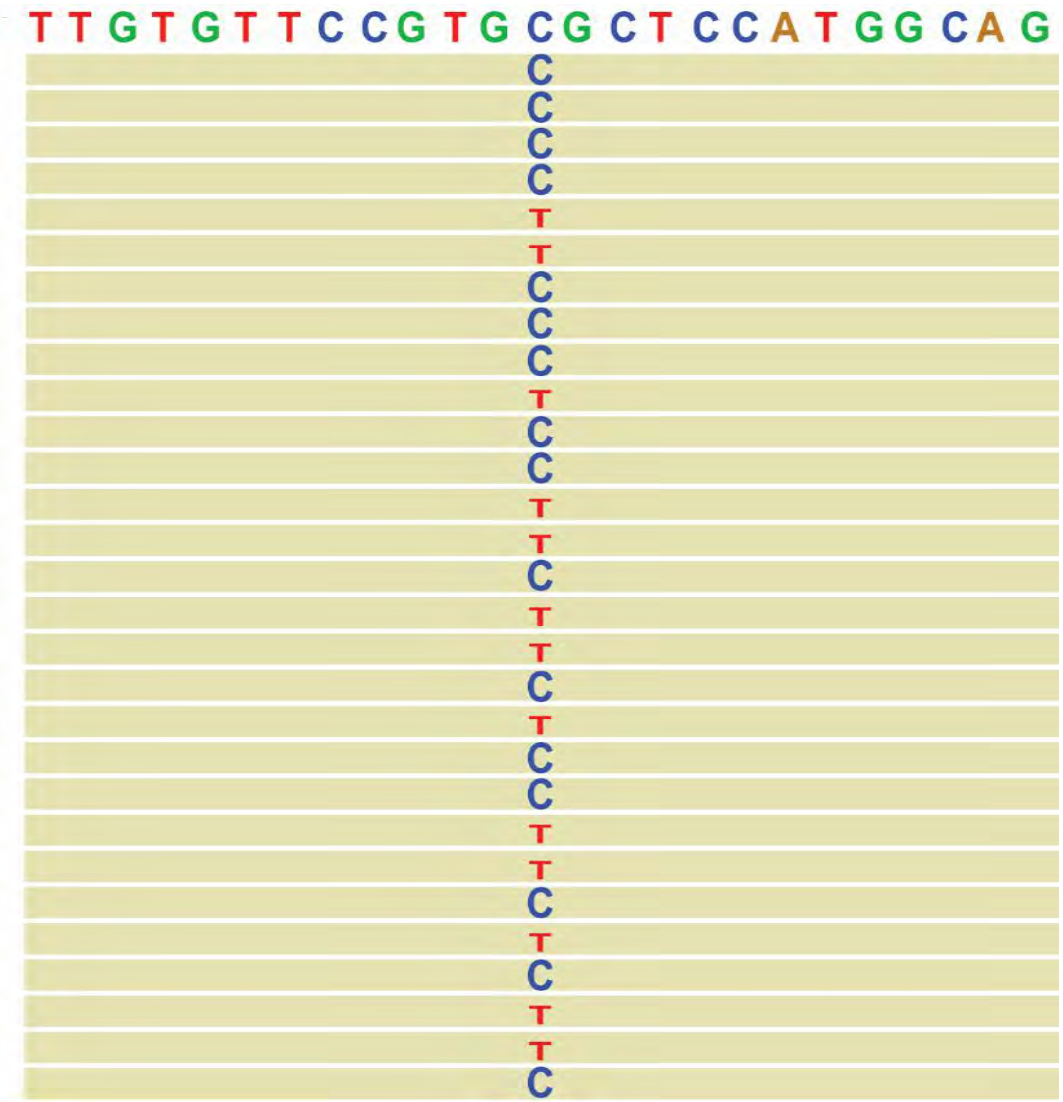
- F Purified virus population displayed CPE plaque morphology.
- Neither virus population’s titer changed substantially over passage.

Alignment of Full-Length Genomes Shows Limited Areas of Diversity



- Repeated regions are less conserved.

Minority Variants within Consensus Sequence



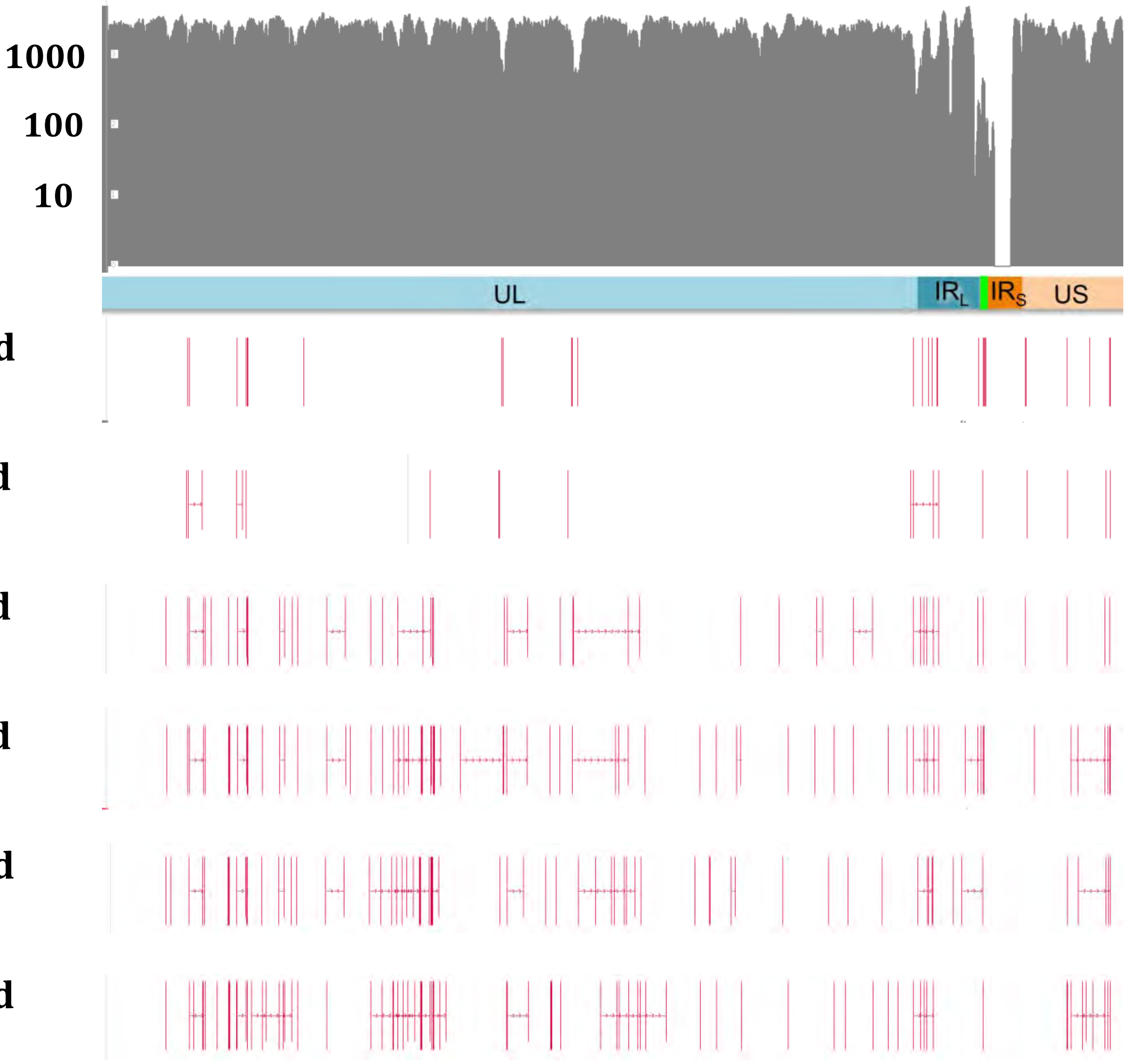
Consensus Sequence

Major Allele (C)

Minor Allele (T)

- Deep sequencing can reveal sequence variation at a sub-consensus level.

Minority Variants in a Mixed Population over Passage

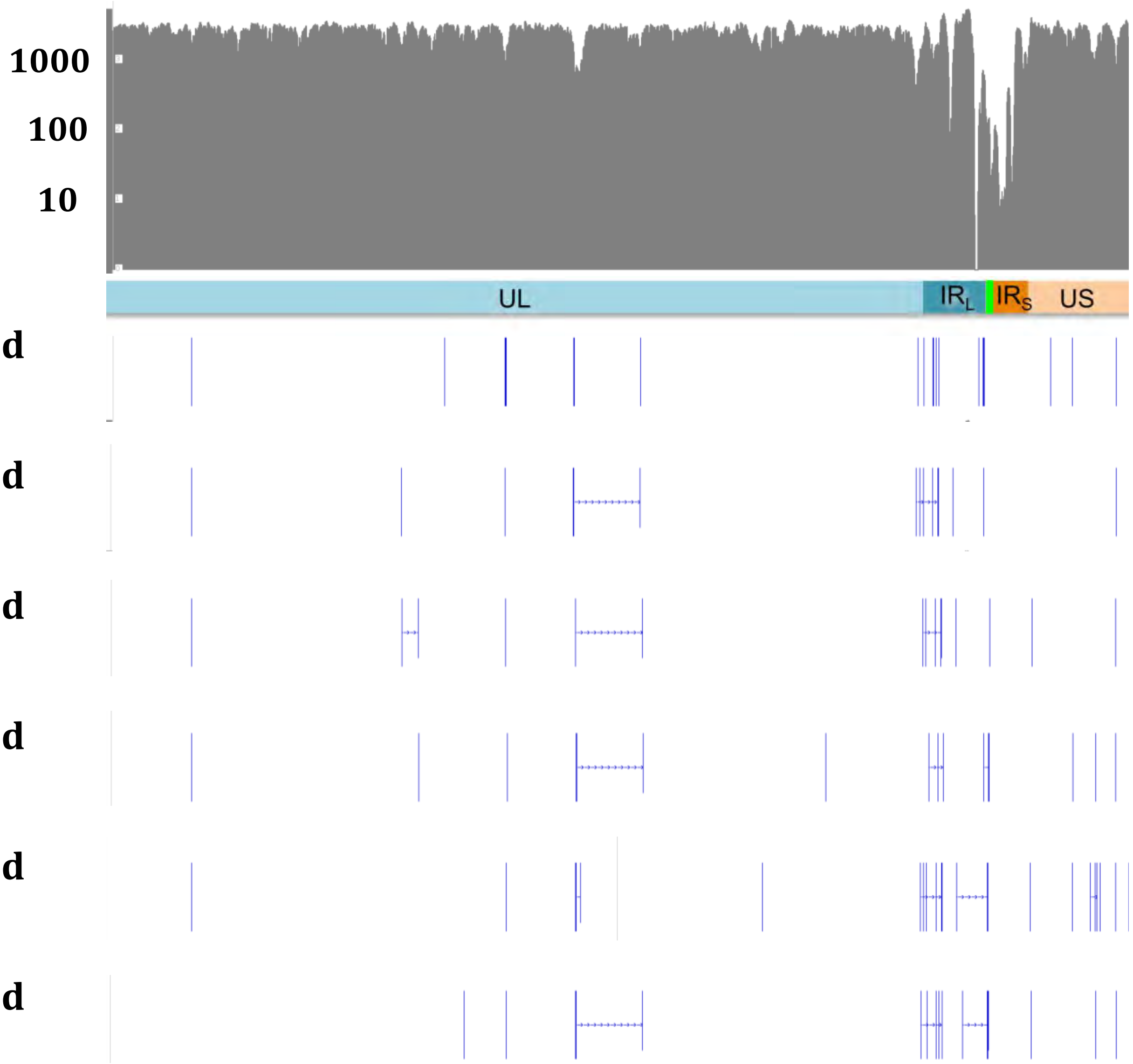


Representative Sequencing Coverage (Read Depth)

Minority Variants (≥ 2%)

N=945 Minority Variants (10 passages)

Minority Variants in a Purified Population over Passage

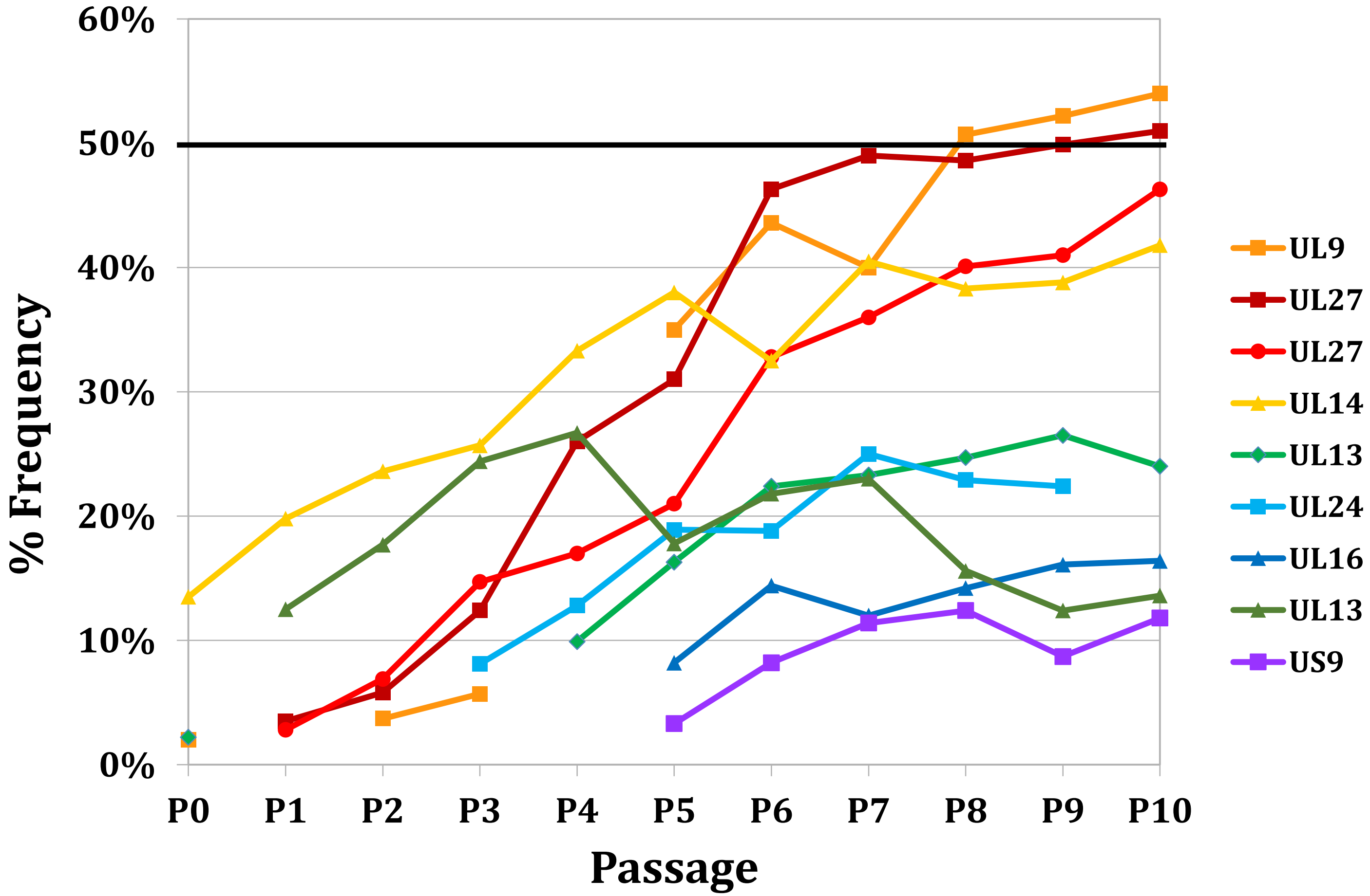


Representative Sequencing Coverage (Read Depth)

Minority Variants (≥ 2%)

N=608 Minority Variants (10 passages)

Minority Variants in Coding Regions Vary in Frequency Over Passage



- This is a small subset of all observed minority variants in coding regions in the mixed population passages.

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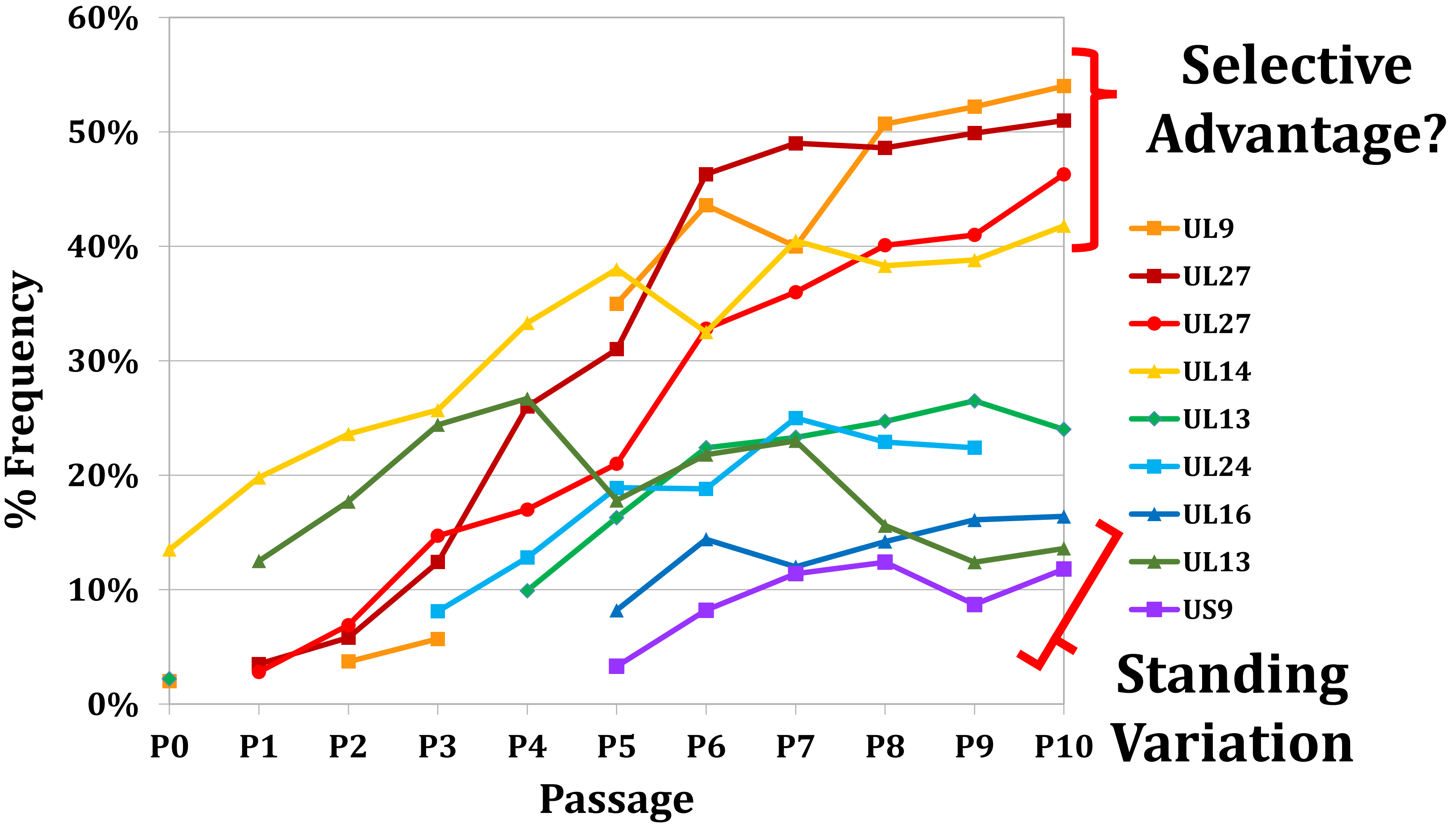

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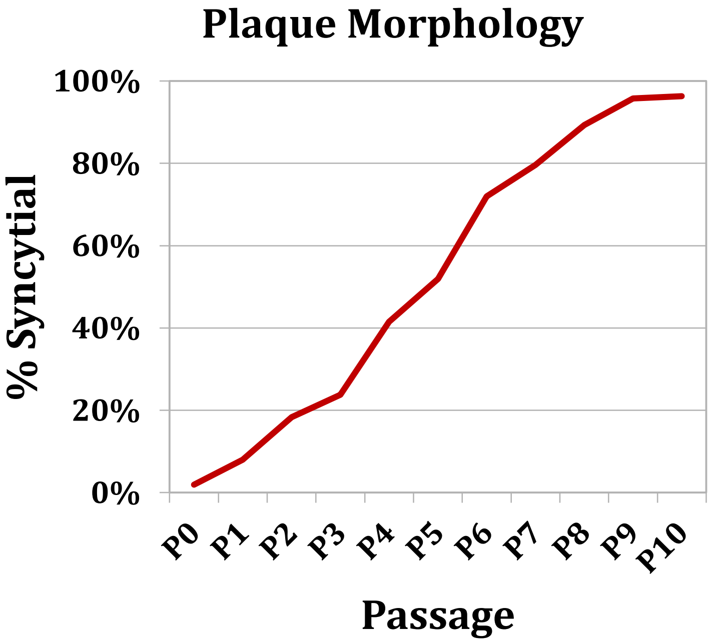
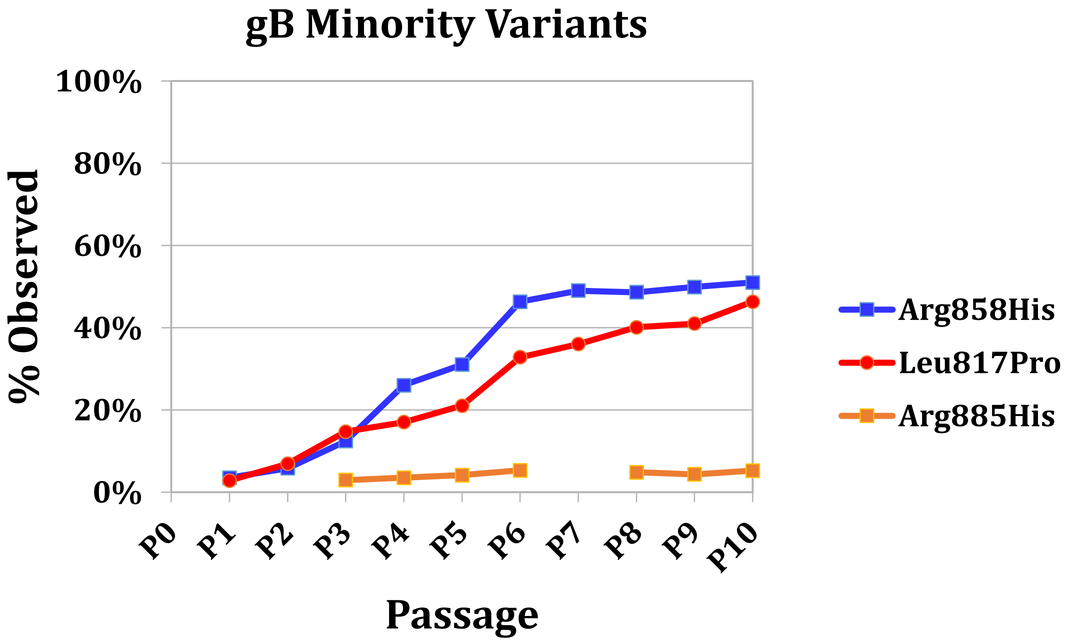
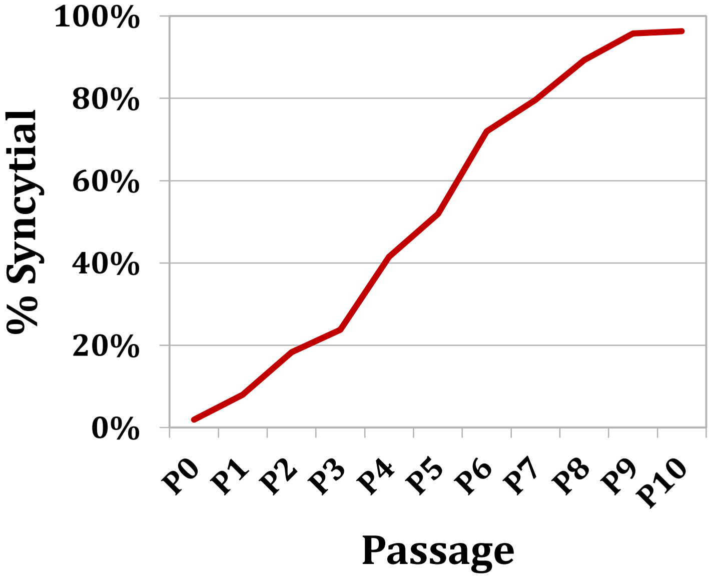
gB Minority Variants Associated with Syncytia


Plaque Morphology

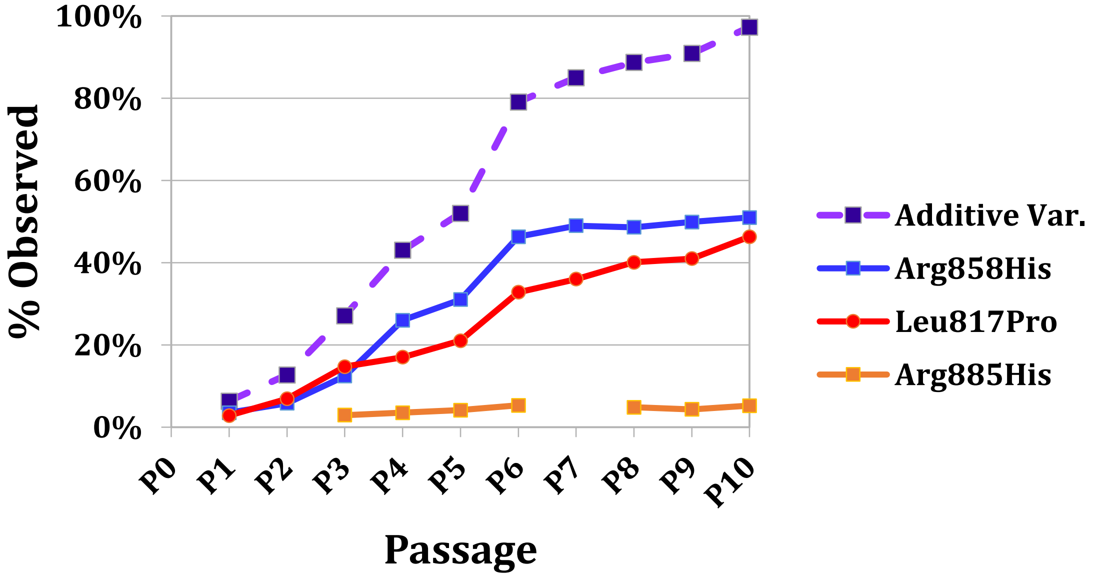

gB Minority Variants

- Mixed population took on a syncytial plaque phenotype over passage.
- Two minority variants in gB increased in frequency over passage.
- Arg858His and Leu817Pro are both known syncytial mutants (Gage et al., JVI 1993).

gB Minority Variants Associated with Syncytia



Plaque Morphology



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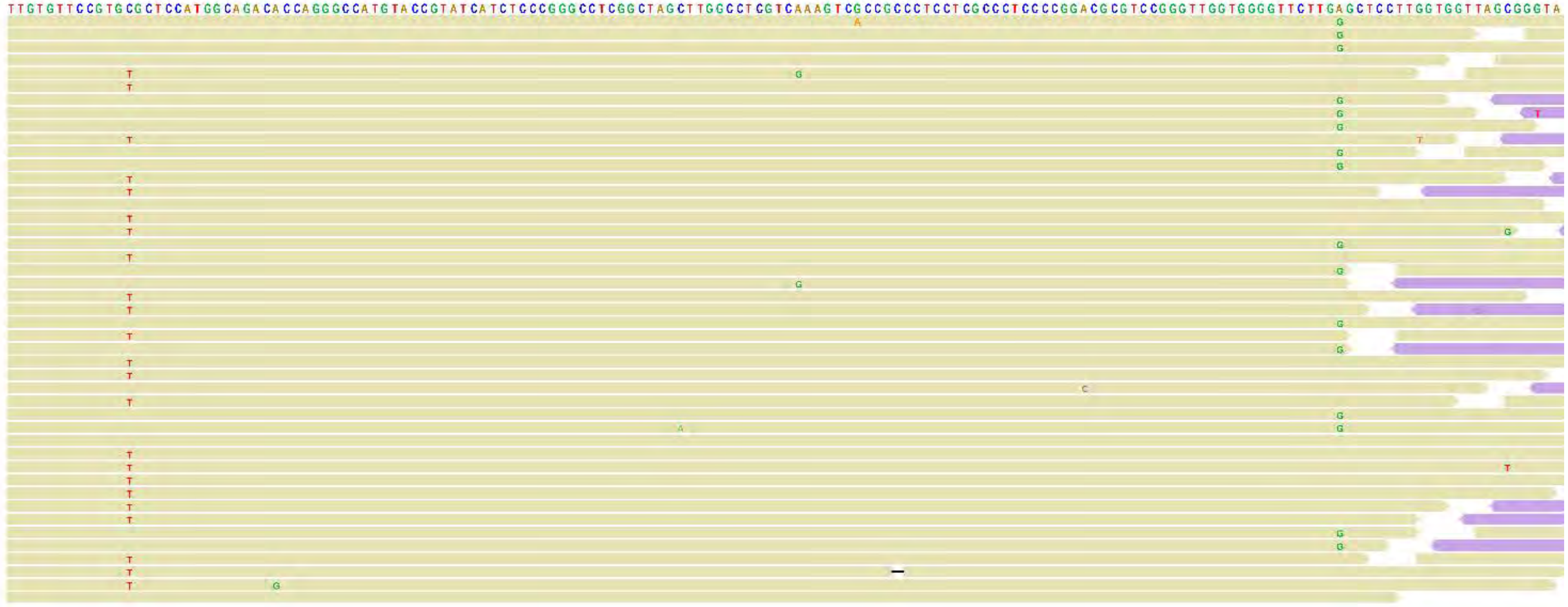
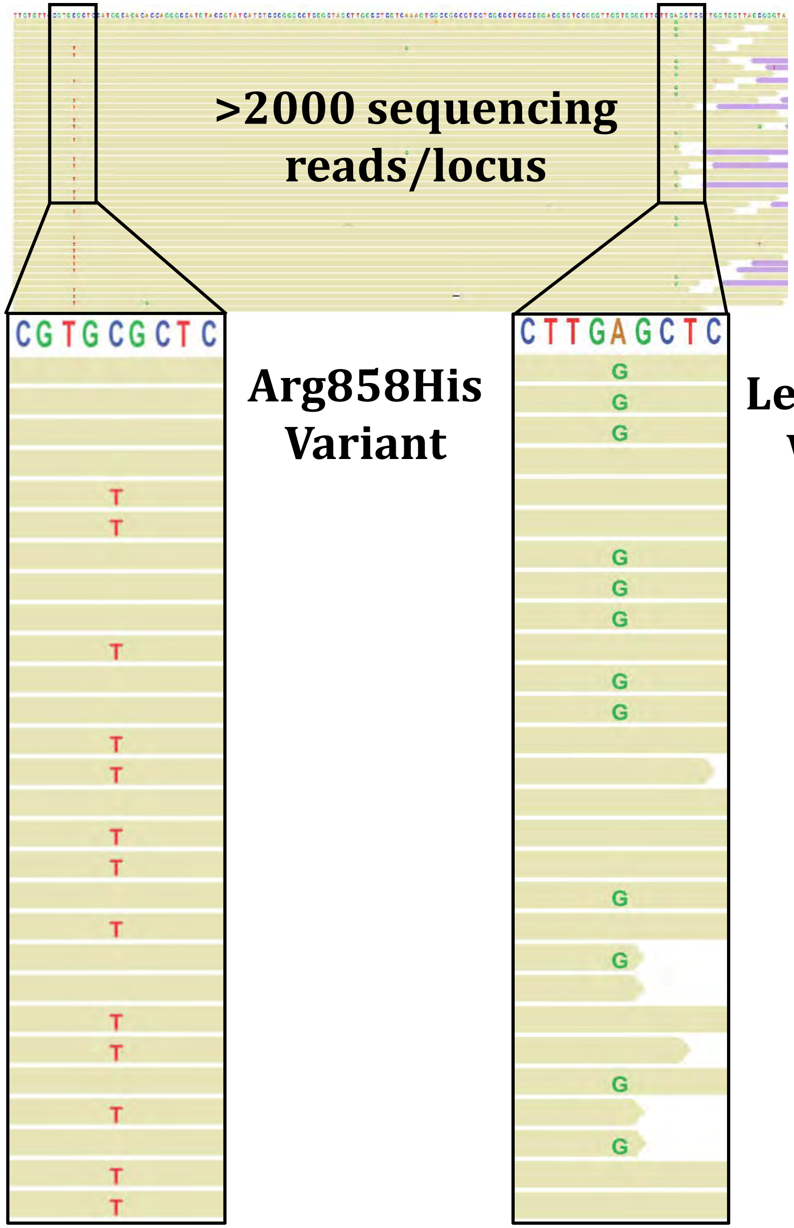


- Variants located close enough to be located within the same sequencing read.
- We can then see how often each variant occurs on a given piece of DNA.

gB Minority Variants Associated with Syncytia



>2000 sequencing reads/locus

Arg858His Variant

Leu817Pro Variant

397 reads span both minority variants

	Reads (P8)	Plaque Morphology (P8)
WT	11%	11% CPE
L817P	37%	89% Syncytial
R858H	52%	
Both	0.002%	

Conclusions

- Minority genetic variants can have major effects on virus biology.

Acknowledgements

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