Sequencing West Nile Virus in Wyoming using Illumina Microbial Amplicon Prep Kit

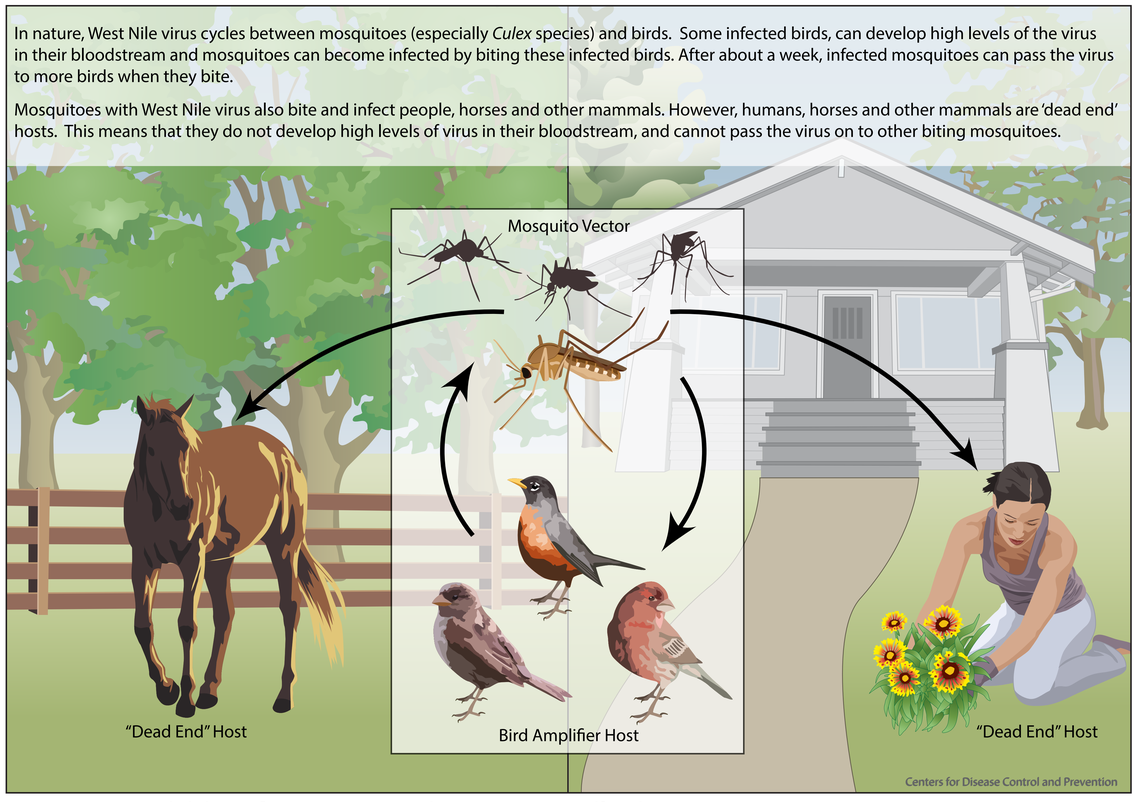
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What is West Nile Virus?

West Nile Virus (WNV) is the leading cause of mosquito-borne disease in the continental United States. While most people infected with WNV remain asymptomatic, rare cases can lead to severe, sometimes fatal, illnesses. There currently is no treatment or vaccine to prevent WNV infection.

West Nile Virus Transmission Cycle



Source: CDC - Division of Vector Borne Diseases - West Nile Virus

WNV in Wyoming



Culex pipiens

Source: Wikimedia Commons

WNV has been detected in each county of Wyoming in which testing was conducted, making it a good candidate for on-going pathogen surveillance in Wyoming. At the Wyoming Public Health Laboratory (WPHL), it is important that we continue to expand surveillance of public health pathogens in Wyoming.



Culex tarsalis

Source: Jim Moore (BugGuide)



Step 1: Collect and grind up mosquitos



Step 2: Test mosquito pools for WNV via PCR



Step 3: Sequence WNV+ pools following iMAP protocols



Step 4: Conduct bioinformatics analysis

Bioinformatics Analysis

For analysis of our WNV sequencing we utilized a few tools. First, we used Bactopia's "clean-yer-reads" workflow to QC our data and followed it up with the Kraken2 Bactopia Tool to extract WNV reads. With the WNV reads we then used iVAR along with a BED file based on primers available from the Grubaugh Lab (see QR code) to generate consensus assemblies based on WNV reference NC_009942.1. Finally, we used a WNV tool available from the Genome Detective web-app to type our consensus assemblies. Each of our samples were typed as Lineage 1A.

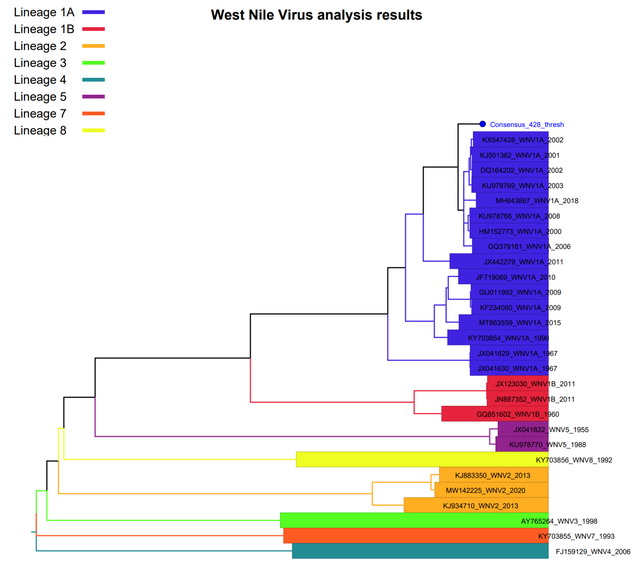


WNV Primers

Sequencing WNV at WPHL

At WPHL, we had established SARS-CoV-2 amplicon sequencing using the COVIDSeq chemistry. Illumina's Microbial Amplicon Prep (iMAP) also utilized the same chemistry as COVIDSeq, so we decided to see if we could repurpose our past experience for WNV sequencing.

To do so we acquired mosquito pool samples from Wyoming that were previously PCR tested to be positive for WNV. A total of 16 different pools were ground up and underwent sequencing using iMAP protocols. For the amplicon sequencing, primers were ordered from IDT using a WNV primer scheme available from the Grubaugh Lab (see QR code below). We then used the Illumina MiSeq for sequencing.



A report generated by WNV Typing Tool for a single sample