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A Novel Approach to Sequencing West Nile Virus Genome using IDT xGen and Illumina MiniSeq.

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Introduction

- West Nile virus (WNV) was introduced in 1999 and has subsequently become the most common identified mosquito-borne illness in the continental United States (CDC, 2023).
- Within the last five years (2018–2022), 8,386 WNV human infection cases were recorded in the US. In addition, 90 WNV human infection cases have been reported in 17 states this year.**
- Nebraska has recorded the 4th highest total number of cases since WNV was introduced into the US
- Monitoring the genetic variability of WNV will allow researchers to elucidate transmission patterns and ultimately incorporate WNV genomics into estimates of human risk
- Understanding virus evolution through time requires an in-depth understanding of genomics.
- This research project aims to develop a more efficient and effective method for sequencing WNV genomes to better understand evolution. In addition, a novel approach was adopted to sequence WNV from mosquito pools collected in Nebraska.**

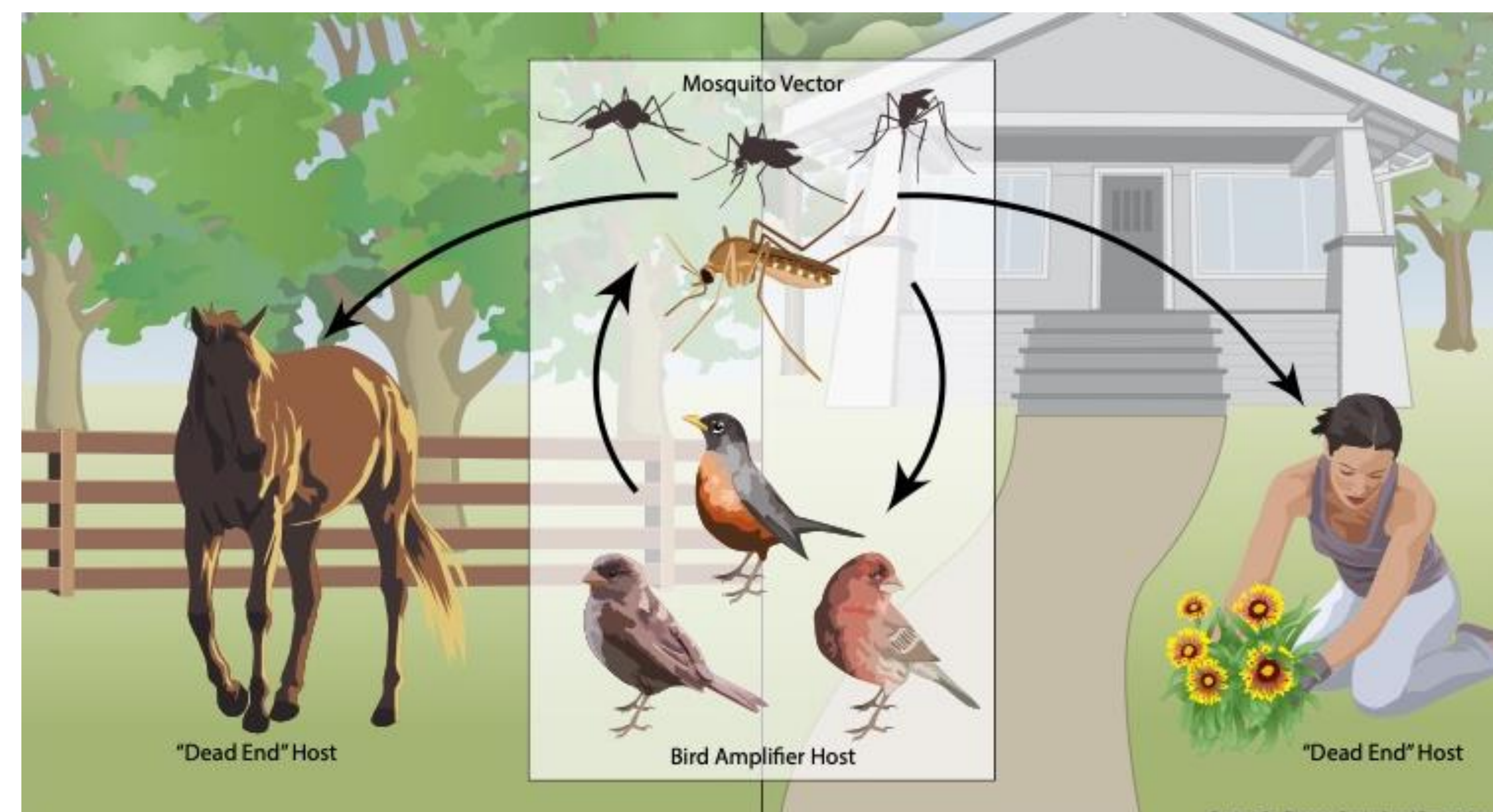
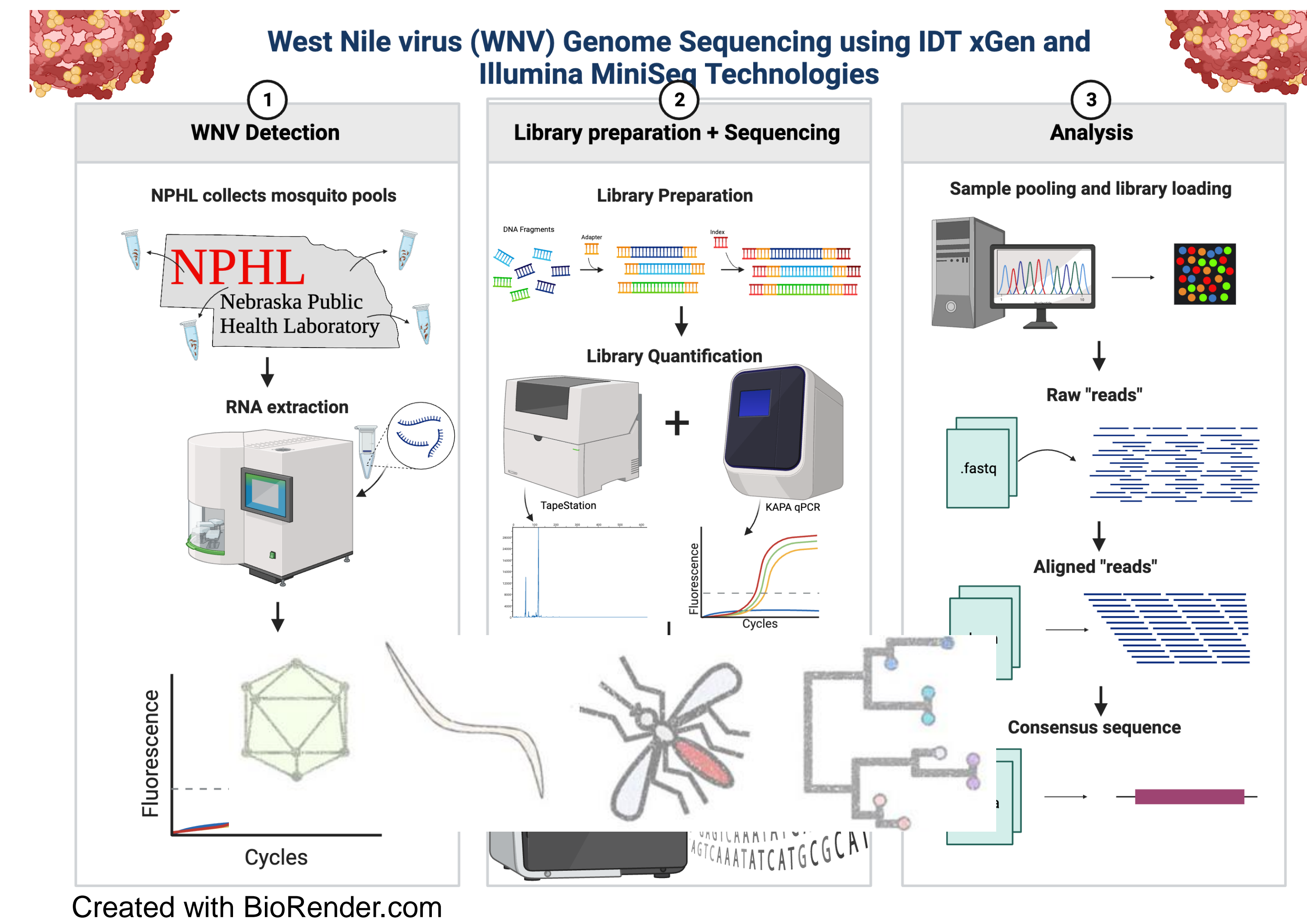
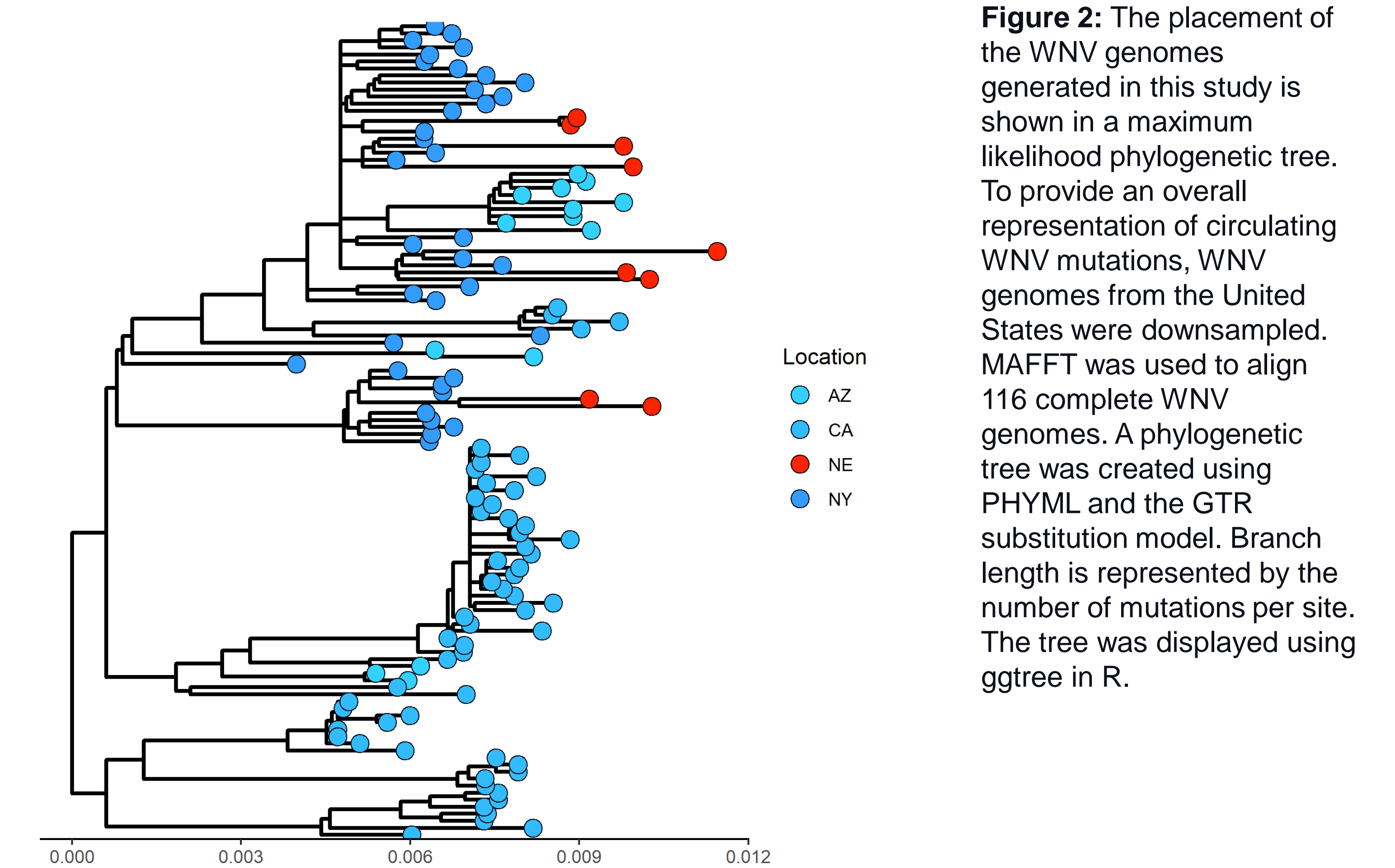


Figure 1: WNV is primarily maintained in an enzootic transmission cycle between *Culex* species mosquitos and birds as vertebrate hosts. Humans and horses, on the other hand, are incidental hosts (also known as dead-end hosts) and cannot be transmitted to another host.

Methods



Result Cont.



Result

Table 1: WNV data analysis using IDT xGen sequencing and Illumina MiniSeq

Sample	Species	Location (County)	Site	Qubit	Average Kapa qPCR Ct Value	#Reads	% Coverage
430	<i>Cx. tarsalis</i>	Wayne	WSC	10.3	7.56	1,256,964	100%
465	<i>Cx. sp</i>	Adams	HMA	3.98	8.02	1,826,169	100%
466	<i>Cx. sp</i>	Adams	HMS	2.94	8.785	2,443,397	100%
500	<i>Cx. tarsalis</i>	Dawson	Canaday	2.86	8.68	2,324,894	99.4%
525	<i>Cx. tarsalis</i>	Dakota	Salvation Army Camp	13.3	8.545	2,008,066	100%
590	<i>Cx. sp</i>	Dodge	Luther Hormel Memorial	10.3	8.31	1,626,101	100%
630	<i>Cx. tarsalis</i>	Lancaster	Sutter	0.218	11.105	1,601,301	100%
673	<i>Cx. sp</i>	Hasting	Good Samaritan Village	0.232	10.805	1,437,861	100%
703	<i>Cx. tarsalis</i>	Dawson	AR House	0.206	12.675	546,718	100%
761	<i>Cx. sp</i>	Hall	Capitol Monitor	1.31	8.33	8,880,670	100%
C1	-	-	-	Out of Range	-	0	0%
C2	-	-	-	0.148	-	7	0%

Notes: The first round of the IDT xGen finished library was recognized as having too high to sequences in Illumina MiniSeq. As a result, the finished library was cleaned with Kapa beads and quantified. This value is used to sequence the final library in the MiniSeq. A Geneious primer was also utilized to run the .bam for DNA data analysis.

Conclusion + Future Direction

- This research project was carried out with the objective of developing an effective approach for sequencing the WNV genome.
- The results of this research suggested that the IDT xGen could potentially be used to sequence WNV from mosquito pools.
- Future research directions include comparing the IDT xGen results against the traditional sequencing approaches technique. It will allow the research to develop an effective approach to better understanding the genomics of WNV.

References



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