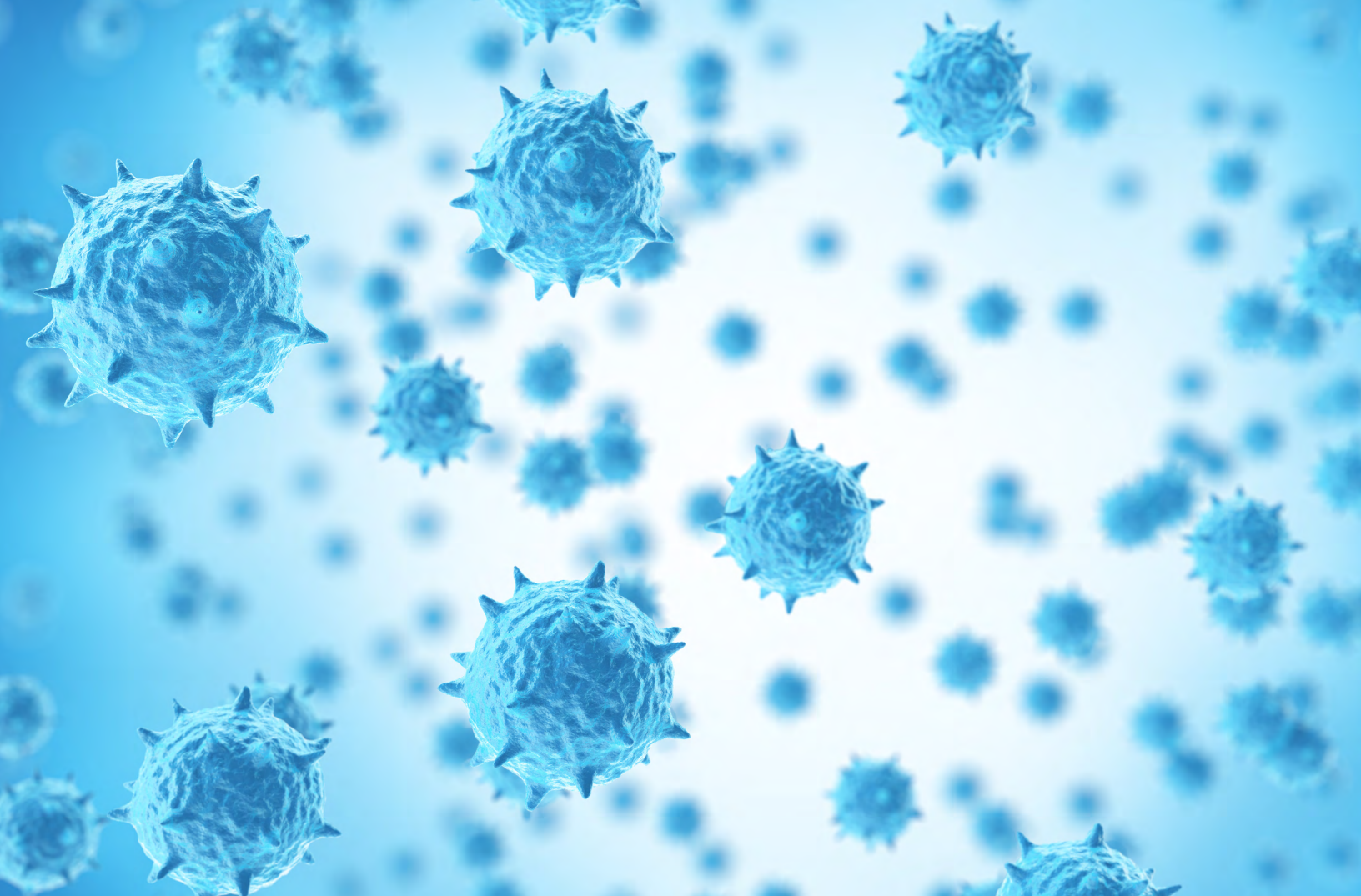




AMPLICON SEQUENCING FOR HIGH-RESOLUTION VIRAL EPIDEMIOLOGY

A GenomeWeb/Integrated DNA Technologies Webinar

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SPEAKER:

Joseph Fauver, PhD

Assistant Professor,
College of Public Health,
Epidemiology Department
**University of Nebraska
Medical Center**



SPEAKER:

Jordan RoseFigura, PhD

Sr. Staff Scientist, Gene Reading
IDT

Webinar Available On Demand

Genomic surveillance can reveal how viruses are introduced, spread, and evolve, but generating complete, accurate genomes from field samples presents practical challenges. Viral loads can vary substantially, and sequence divergence can interfere with primer binding, causing amplicon dropout and leaving gaps that limit downstream epidemiological analysis.

In this GenomeWebinar sponsored by Integrated DNA Technologies (IDT), Joseph Fauver, an assistant professor in the University of Nebraska Medical Center's College of Public Health, discussed his laboratory's use of amplicon sequencing to study West Nile virus (WNV) transmission in Nebraska, including a custom IDT xGen assay designed to generate complete viral genomes from mosquito pools. IDT senior staff scientist Jordan RoseFigura then examined the technology behind the xGen Amplicon workflow and presented data demonstrating its performance with other variable viral genomes, including SARS-CoV-2 and HIV.

Using viral genomes to understand transmission

For Fauver, West Nile virus provides an especially relevant target for genomic epidemiology. Data presented during the webinar showed that WNV incidence is particularly high across the Great Plains. Since 2001, Nebraska has recorded 4,482 cases, placing it behind only California, Colorado, and Texas.

Sequencing WNV genomes can provide information that conventional surveillance alone cannot. Genomic data can help researchers investigate viral introductions and spread, track changes in the frequency of different lineages, and monitor individual single-nucleotide polymorphisms. The objective is not simply to sequence a virus, Fauver explained, but to obtain genomes complete enough to distinguish closely related viruses and reconstruct their relationships.

That makes sequencing quality particularly important. Amplicon-based methods rely on primers binding to specific regions of the viral genome. When a mutation occurs at a primer-binding site, amplification can fail, resulting in amplicon dropout. For epidemiological investigations, those missing regions can translate into missing information.

Fauver and his colleagues therefore evaluated a custom IDT xGen Amplicon assay as a way to generate complete WNV genomes from mosquito pools and compared the resulting sequences with other approaches.

Building a custom xGen assay for WNV

The xGen Amplicon workflow uses multiplex PCR followed by adapter attachment and indexing PCR to create dual-indexed amplicon libraries. An optional normalization step uses Normalase chemistry to normalize the resulting libraries before pooling. The workflow presented in the webinar included approximately 70 minutes for multiplex PCR, 35 minutes for adapter attachment and indexing PCR, and 40 minutes for optional library normalization.

One feature of the technology that is particularly relevant to viral sequencing is the formation of what IDT calls “super amplicons.” In a conventional tiled amplicon workflow, a variant occurring beneath a primer-binding site can prevent an intended amplicon from forming. That can eliminate coverage of the region and potentially prevent another nearby variant from being observed.

In IDT’s one-tube workflow, primers can also generate longer, overlapping super amplicons. As illustrated during the presentation, these additional products can span a region in which an intended amplicon has dropped out, maintaining coverage and allowing variants in that region to remain detectable.

That feature became important when Fauver’s group tested the method against genetically diverse WNV samples. Their results indicated that the IDT xGen approach was less susceptible to dropout, including across viral genomes with up to 5 percent nucleotide divergence.

The researchers also compared consensus genomes generated using different sequencing approaches. In a set of 10 matched 2022 samples prepared using a Primal Scheme with Illumina sequencing, the IDT xGen workflow with Illumina sequencing, and the IDT xGen workflow with Oxford Nanopore Technologies’ MinION platform, the consensus sequences showed no nucleotide differences attributable to sequencing approach.

Together, the results supported the group’s use of the custom assay to generate the complete, accurate WNV genomes required for higher-resolution epidemiological analysis.

Turning sequence data into a picture of WNV transmission

With reliable genomes in hand, Fauver’s team could move from assay performance to epidemiology.

Their phylogenetic analyses placed Nebraska WNV genomes within the broader diversity of WNV circulating in North America. The resulting genomic relationships enabled the researchers to investigate how viruses detected in Nebraska were related to one another and to characterize transmission patterns that would be difficult to discern from case counts alone.

This illustrates the larger value of high-resolution viral sequencing: Small genetic differences become markers that researchers can use to reconstruct viral movement and transmission. The approach can help distinguish between viral introductions and subsequent local spread and provide a more detailed view of how circulating viral populations change over time.

The analysis also reinforced why genome completeness matters. When the differences separating epidemiologically relevant samples may be small, systematic gaps created by amplicon dropout can reduce the resolution available for phylogenetic inference. A sequencing method that tolerates some divergence from the sequence used for primer design can therefore be particularly useful for genomic surveillance of an evolving virus.

Fauver also showed that WNV genome completeness was associated with Ct value, underscoring the challenge posed by samples containing different amounts of viral material.

Extending xGen Amplicon beyond West Nile virus

Following Fauver's presentation, RoseFigura broadened the discussion to IDT's xGen NGS tools for viral research and showed how the same underlying amplicon strategy has been evaluated with other viruses.

With the xGen SARS-CoV-2 Amplicon Panel, IDT demonstrated how super amplicons can help preserve coverage as a virus accumulates sequence variation. A single primer set designed against the original Wuhan-1 reference generated 99.7 percent genome coverage, using 345 contiguous primer pairs in a single multiplex PCR. The same design maintained coverage across Alpha, Delta, and Omicron lineages without requiring new primer designs as those variants emerged.

The SARS-CoV-2 data also examined performance across viral inputs and sequencing depths. The results showed high genome coverage across a wide range of conditions, including low viral-copy inputs. The presentation highlighted the ability to obtain coverage from as few as 10 viral copies, while also demonstrating strong coverage at relatively modest read depths for samples containing more viral material. RoseFigura noted that the panel could maintain full genome coverage from 50,000 reads depending on viral input, making the approach applicable to samples across a range of Ct values when lineage information is needed.

IDT also tested the approach against an even more variable target: HIV. The company designed a densely tiled panel against the HXB2 reference sequence comprising 109 amplicons, with an average amplicon size of 102 base pairs and no redundant or degenerate primer sequences. It then evaluated amplification using an HIV genome construct with only 93 percent identity to the reference used for primer design.

The experiment also provided a way to examine the contribution of super amplicons directly. Libraries generated with the xGen Amplicon workflow were sequenced on Illumina instruments using paired-end 150- and 250-base reads and with a workflow adapted for Oxford Nanopore sequencing. Coverage increased with longer Illumina reads, consistent with longer reads capturing more of the super amplicons spanning difficult regions. Long-read Oxford Nanopore sequencing made even more of those products accessible.

The HIV experiment is an important complement to the WNV and SARS-CoV-2 work because it tests the underlying approach against substantial sequence divergence. Rather than relying on redundancy or degeneracy in primer design, the workflow uses overlapping amplification products to help maintain coverage when individual primer sites are disrupted.

Customizing NGS approaches for viral research

RoseFigura also discussed IDT options for researchers whose projects require a different targeting strategy. IDT's NGS Design team develops custom hybridization capture panels, amplicon panels, blocking oligos, adapters, and indexing primers, allowing researchers to tailor enrichment strategies to the organism and scientific question.

For custom hybridization capture panels specifically, IDT described several design-service pathways that differ in turnaround time and level of consultation. These range from more transactional web and regular design options to a VIP design service intended to provide more specialized consultation and design feedback. The broader point is that viral sequencing projects may require different targeting strategies depending on whether researchers are studying known pathogens and variants or attempting to characterize broader viral populations and novel genomes.

Researchers interested in discussing an NGS project with IDT can [request an NGS consultation](#). More information about designing a custom hybridization capture panel is also available at IDT's [xGen Custom Hyb Panels consultation](#).

Matching sequencing technology to the epidemiological question

Taken together, the data presented in the webinar illustrate both the promise and the practical demands of amplicon sequencing for viral epidemiology. Complete viral genomes can reveal introductions, lineage dynamics, individual variants, and transmission relationships, but the quality of those conclusions depends on generating sequence data that remain reliable despite variation in viral abundance and genome sequence.

Fauver's WNV work demonstrated how a custom xGen assay could be applied to real-world mosquito surveillance. The approach generated accurate consensus genomes across Illumina and MinION sequencing, while its reduced susceptibility to amplicon dropout allowed the researchers to work across WNV genomes showing up to 5 percent nucleotide divergence. Those genomes could then be used to investigate WNV transmission in Nebraska at a resolution that epidemiological surveillance alone could not provide.

RoseFigura's SARS-CoV-2 and HIV examples provided additional evidence for the underlying principle. Super amplicons can preserve coverage when sequence variation interferes with individual primer-binding sites, while longer sequencing reads can capture more of those products. Meanwhile, the relatively short xGen Amplicon workflow and optional Normalase-based normalization offer a path toward scaling the approach for higher-throughput research studies.

For researchers conducting genomic surveillance, the implication is that sequencing depth alone cannot compensate for regions that fail to amplify. Designing an assay capable of maintaining coverage as a virus evolves can help ensure that the genetic differences most relevant to epidemiological investigation remain visible.

[View Publication:](#)

[Genomic Characterization of a Severe West Nile Virus Season Using Single-Reaction Amplicon Sequencing](#)