Genomic Surveillance of West Nile Virus in the United States Using Blood Donations: Methods and Implementation

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32nd IPFA/PEI International Workshop on Surveillance and Screening of Blood-borne Pathogens

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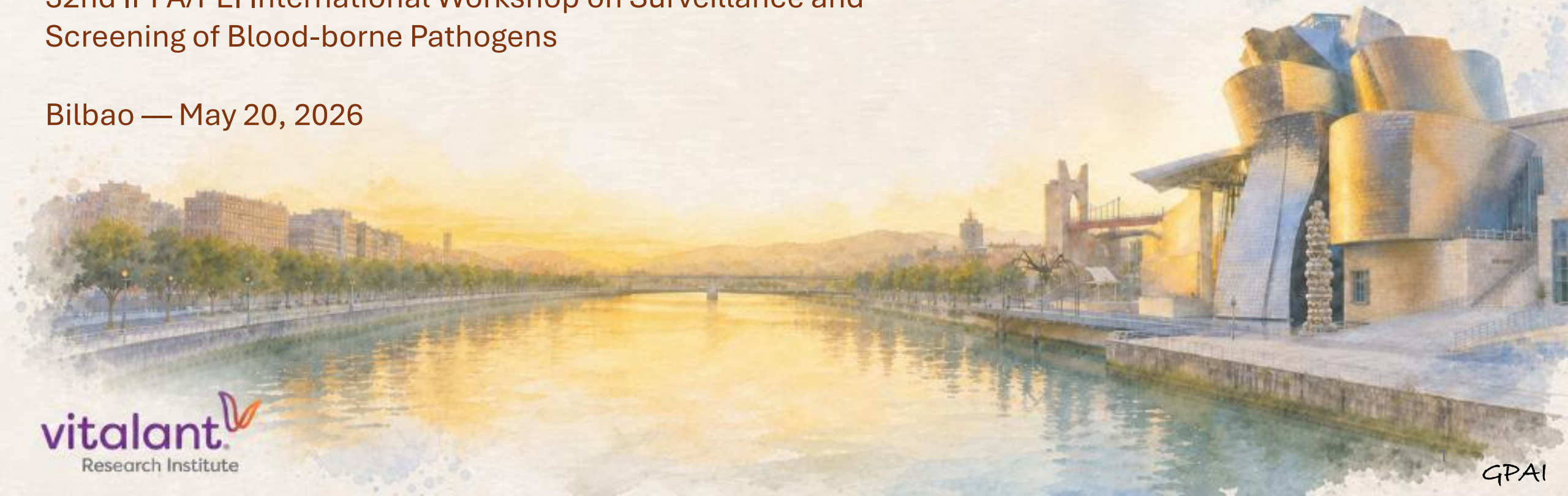

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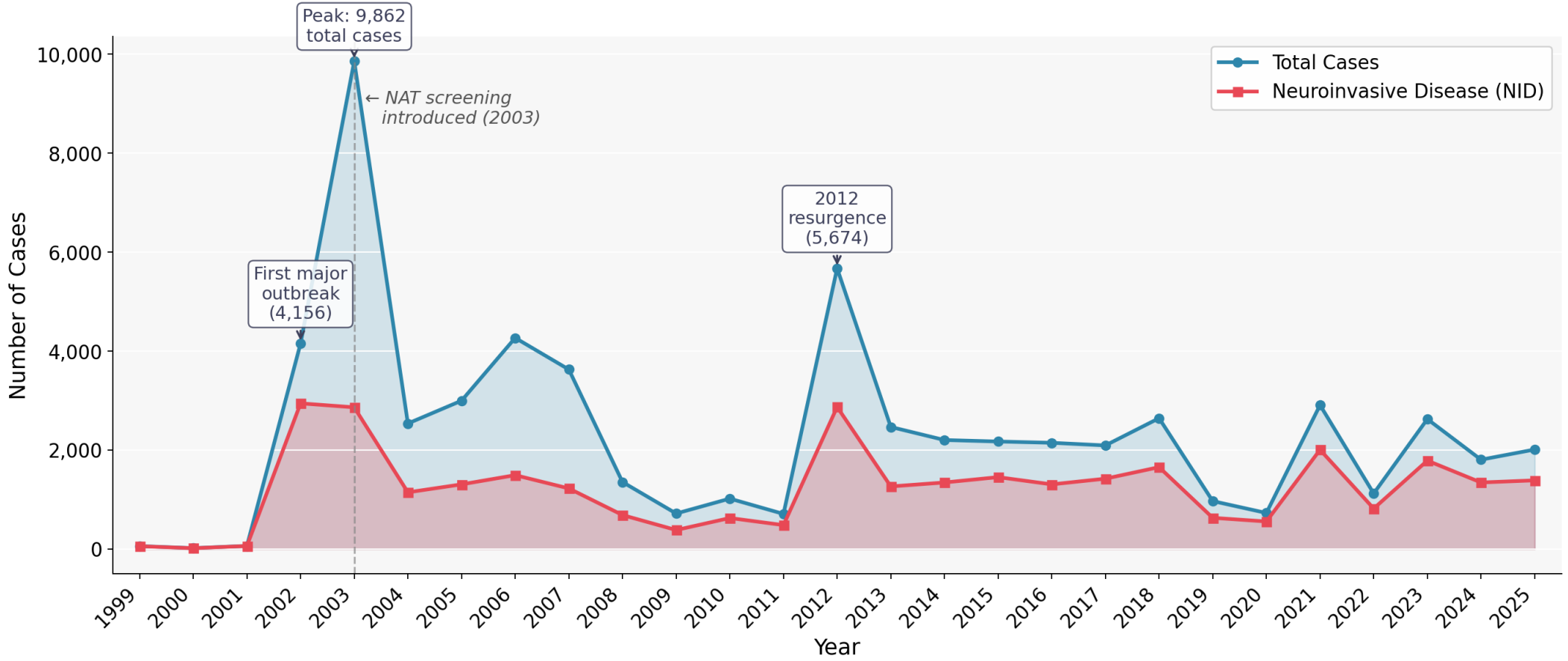
West Nile virus in the United States: from introduction to endemicity



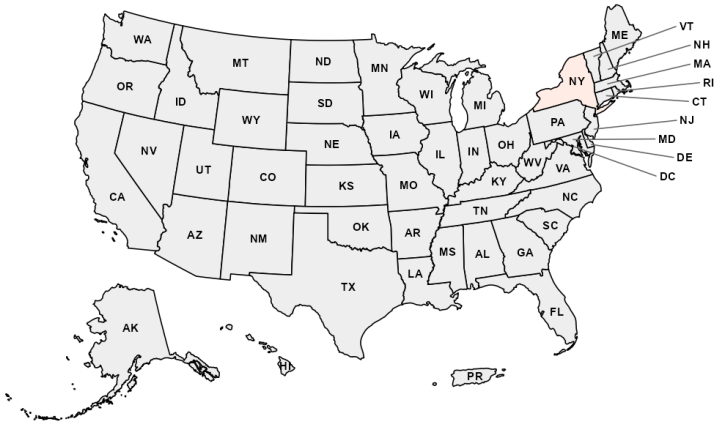
- Since its introduction in New York in 1999, WNV has become the leading cause of mosquito-borne disease in the contiguous United States.
- Large epidemic waves occurred in 2002–2003 and again in 2012.
- Neuroinvasive disease accounts for roughly half of reported cases, but milder disease is substantially under-ascertained in passive surveillance.

Data source: ArboNET. Data are current as of January 13, 2026

Geographic expansion and establishment of West Nile virus in the U.S.

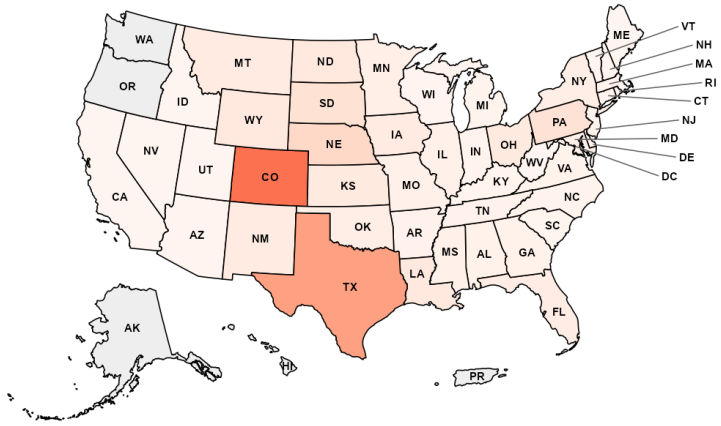
1999

Introduction — 1 state, 59 NID



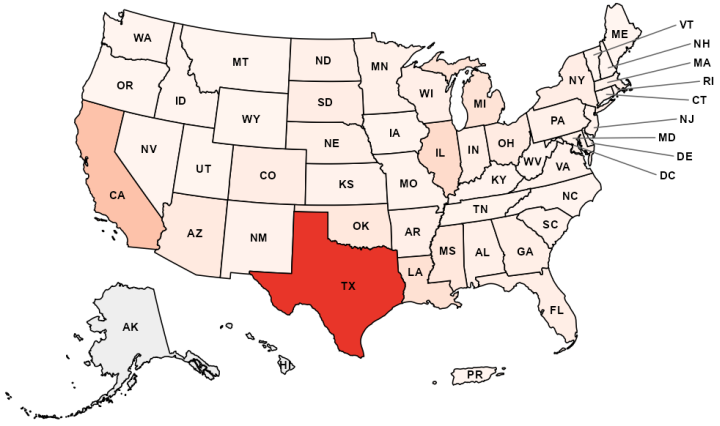
2003

Epidemic peak — 43 states, 2,866 NID



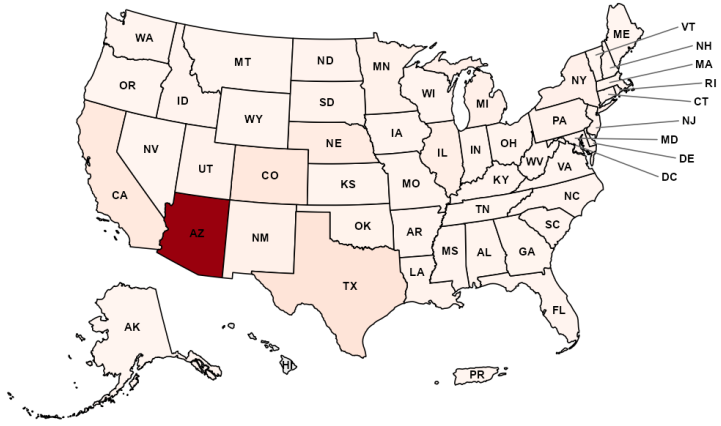
2012

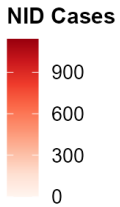
Resurgence — 47 states +DC +PR, 2,873 NID



2021

Recent season — 49 state, +DC +PR, 2,008 NID





- **1999:** initial introduction detected in New York
- **2003:** rapid expansion across much of the U.S., with major amplification in the Great Plains and western states
- **2012:** large nationwide epidemic
- **2021:** Maricopa County, AZ, accounted for 51% of U.S. WNV cases —the largest single-county outbreak on record (956 NID, 101 deaths) JAMA. 2025 Aug 19;334(7):618-628).

WNV remains capable of substantial local resurgence

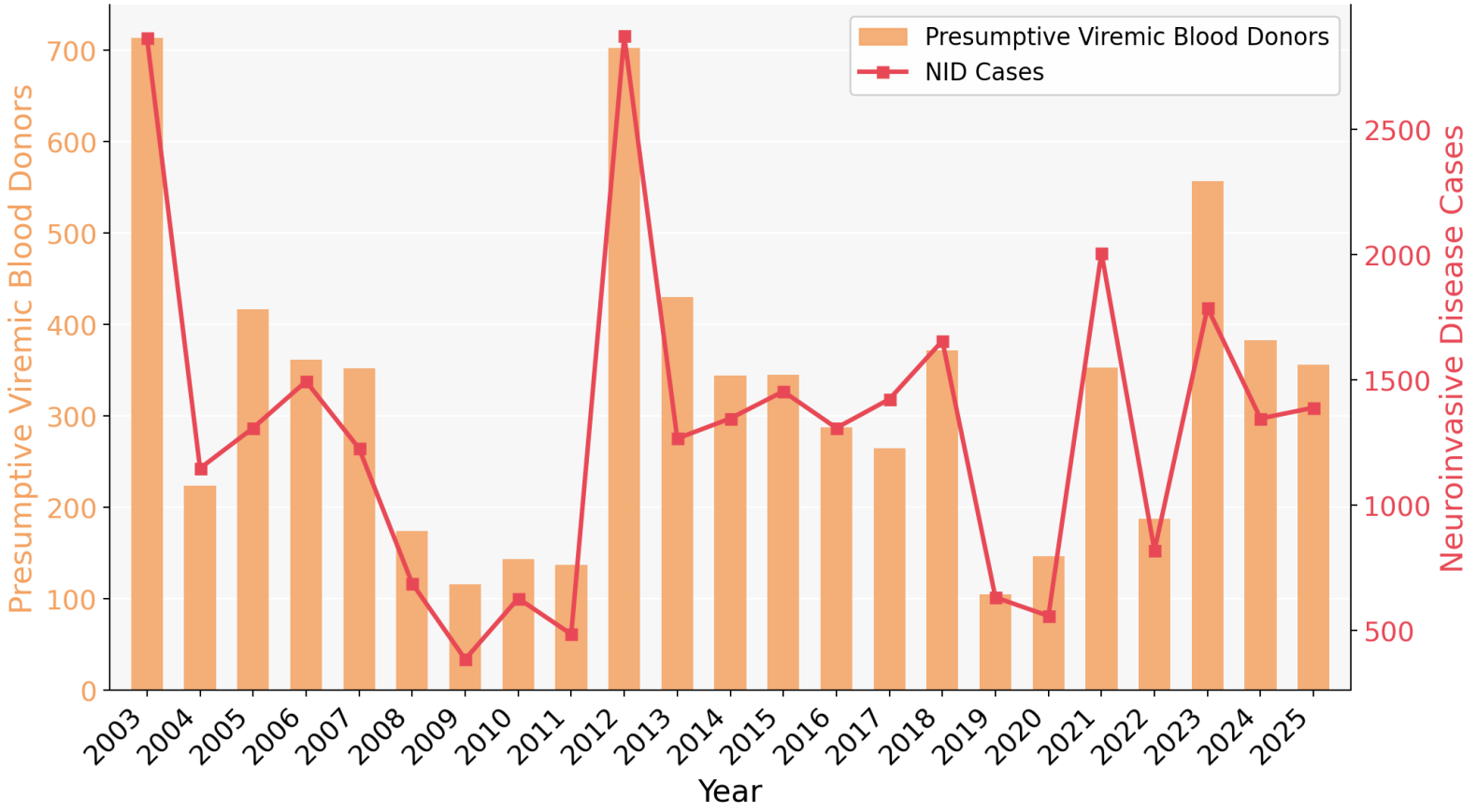
Source: CDC ArboNET, 1999–2023. NID = Neuroinvasive Disease cases.
Jurisdictions include 50 states, DC, and Puerto Rico. Areas with no reported cases shown in grey.

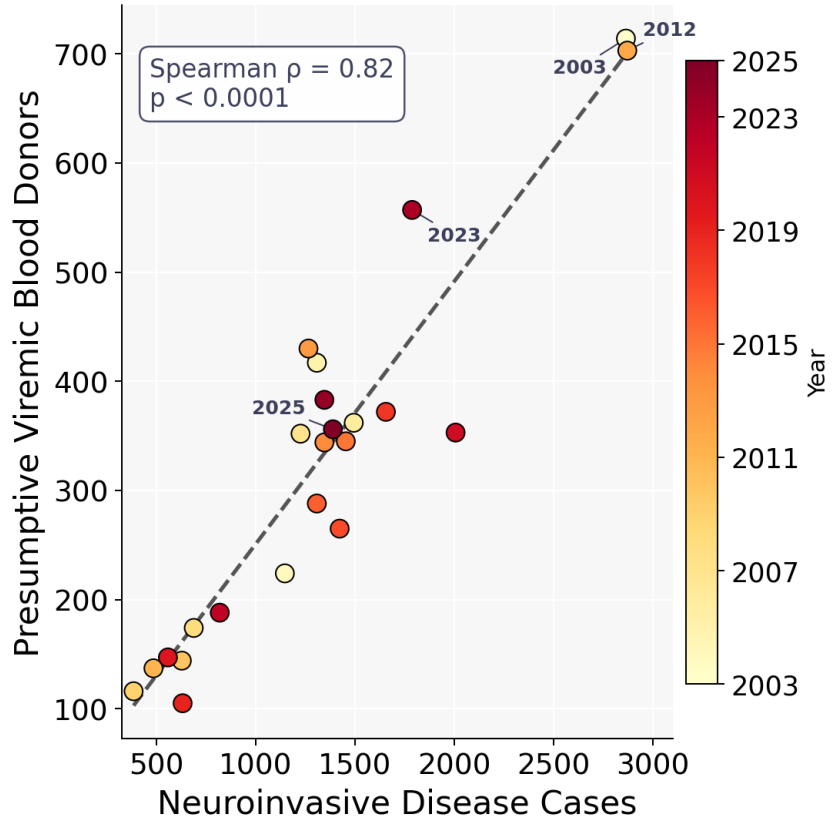
WNV and blood safety in the United States: transfusion transmission and NAT response

- **2002**: 23 confirmed transfusion-transmitted infections linked to 16 viremic donors established WNV as a blood-safety threat [1]
  - 43% immunocompromised (transplant or cancer)
  - 35% aged ≥70 years
- Even when donor viremia was low, recipients showed prolonged viremia, delayed or attenuated antibody response, and detectable WNV RNA in serum or CSF
- **2003**: implementation of nucleic acid testing (NAT) screening
- **Current screening strategy**: all donations undergo licensed WNV NAT; donations are screened by minipool-NAT at baseline, when local activity reaches a predefined trigger—commonly 1 presumptive viremic donation—the affected collection area switches to individual donor-NAT

[1] *N Engl J Med*. 2003;349:1236–45

Blood donor WNV detections closely track neuroinvasive disease burden





Since 2003, **7,476** presumptive viremic blood donors (PVBDs) have been detected through NAT screening across 23 transmission seasons.

Data source: ArboNET. Data are current as of January 13, 2026

Why genomic surveillance matters for WNV epidemiology and blood safety

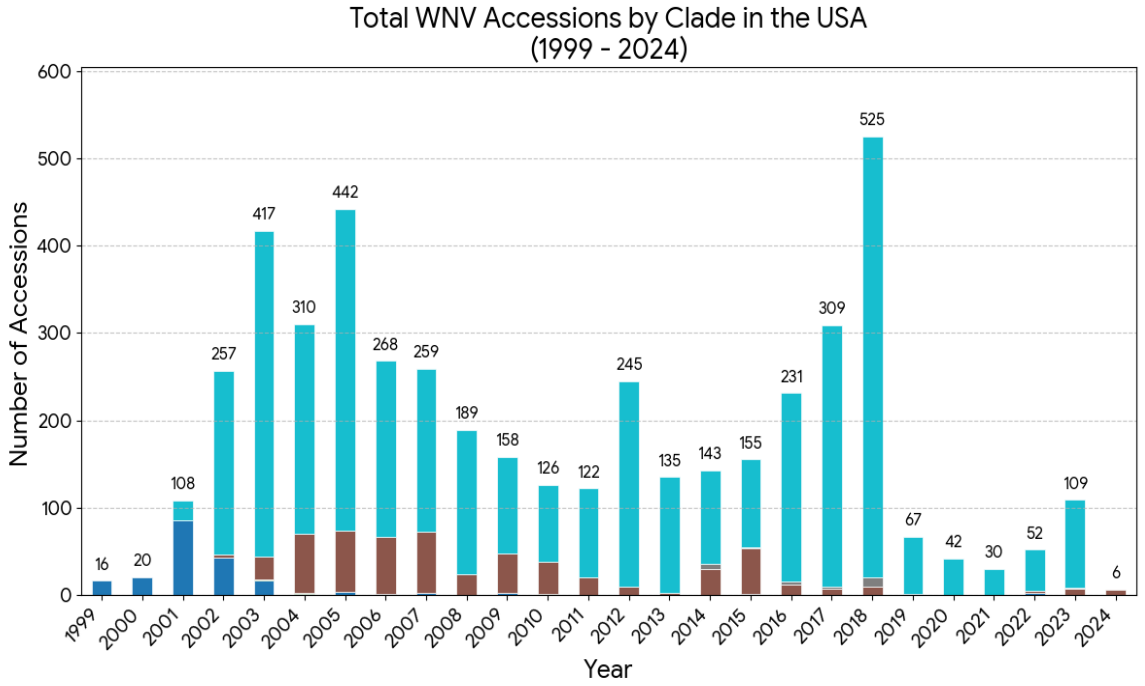
Resolve introduction and spread — Identify lineage turnover, co-circulation, and phylogeographic movement that case counts alone cannot resolve.

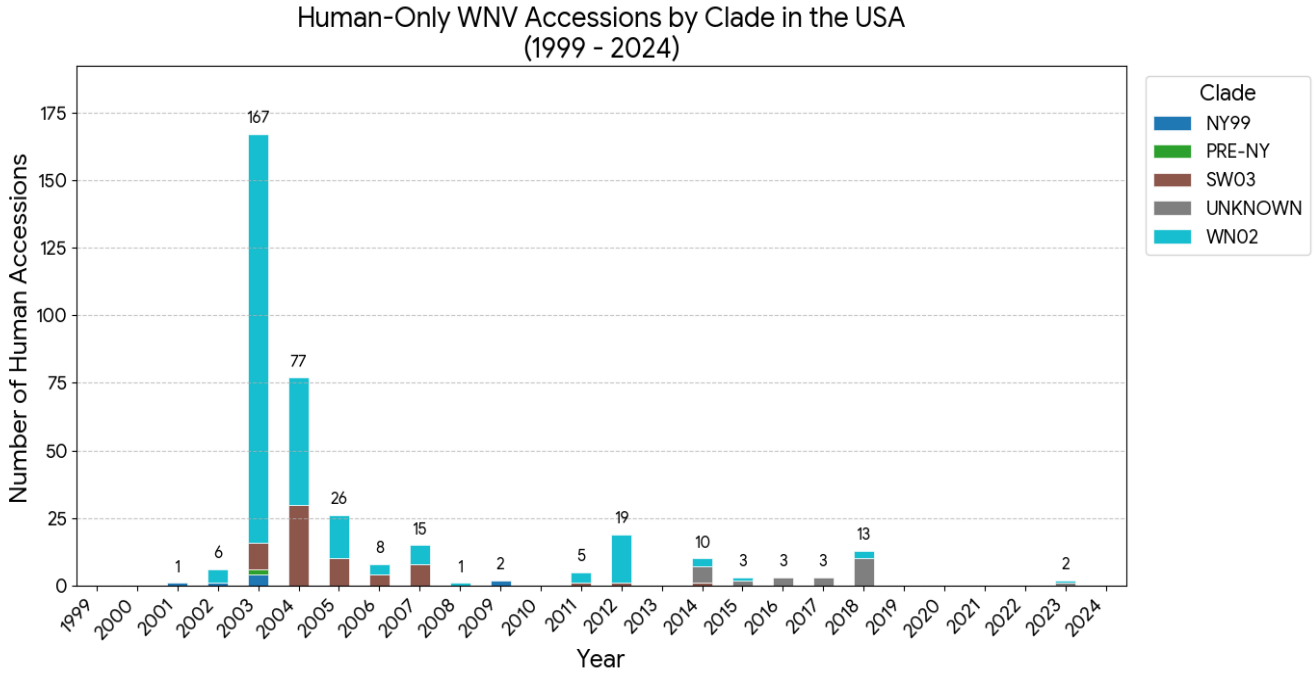
Detect cryptic persistence — Reveal local maintenance, including overwintering or sustained low-level transmission between outbreak seasons.

Track viral evolution — Monitor variant turnover across seasons and regions to understand how circulating WNV populations change over time.

Safeguard NAT screening — Monitor primer- and probe-binding regions for sequence changes that may reduce assay sensitivity.

Declining U.S. WNV genomic surveillance since 2019 and persistent scarcity of human genomic data





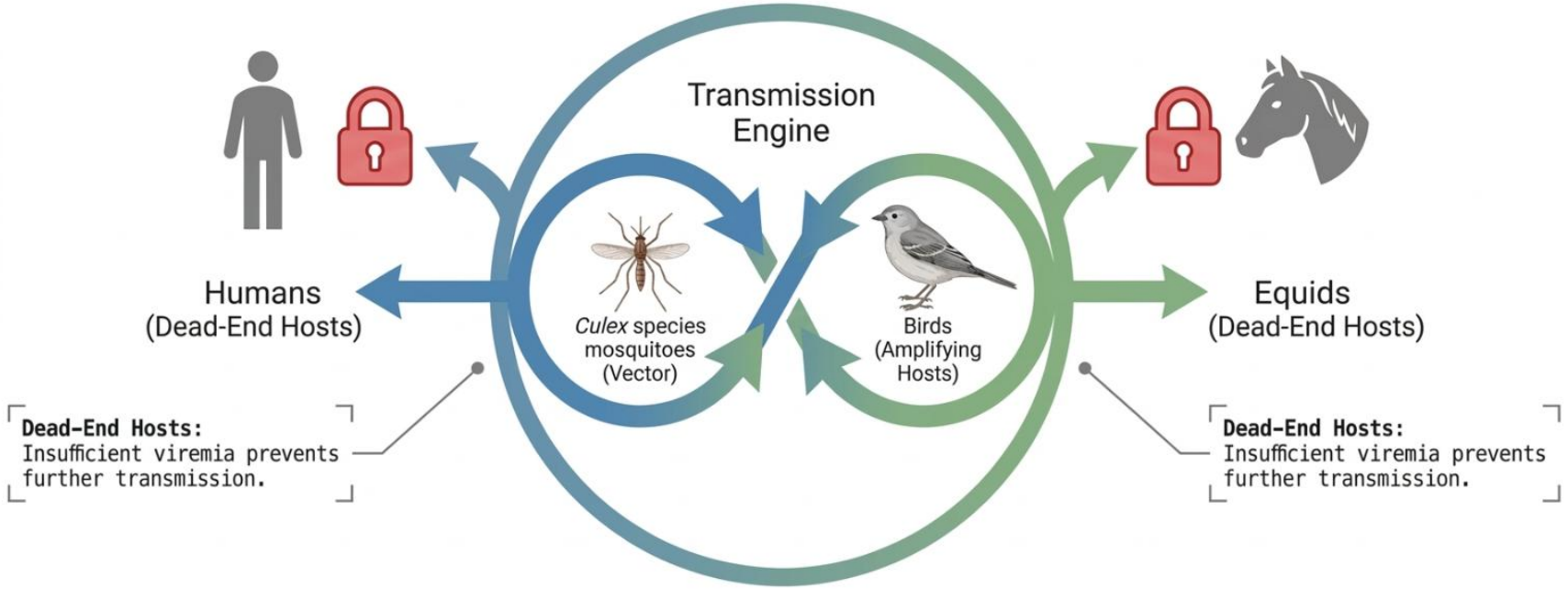
- Public U.S. WNV genome submissions peaked in 2018 but have declined sharply in recent years.
- Human-derived U.S. whole genomes remain especially scarce across most transmission seasons.
- This limits detection of lineage turnover, regional persistence, and unexpected human infections.

Source: Publicly available WNV genomes represented in the Nextstrain WNV build (GenBank-derived metadata; accessed March 2026)

Why human-derived WNV genomes matter

Human sequencing complements enzootic surveillance

- Mosquito and bird surveillance define circulation in the transmission cycle; human-derived genomes show the viruses associated with spillover into humans
- Human-derived genomes define the subset of WNV diversity observed in human infection, which may differ from the broader viral population circulating in vectors and birds



Schematic generated with GPAI

Why NAT-positive blood donors are uniquely valuable for WNV genomic surveillance

- **Early access to human infection**

WNV viremia is brief and often precedes symptom onset; donor screening captures virus during the acute viremic phase.

- **Captures infections missed clinically**

Most WNV infections are asymptomatic; donor screening identifies infections that would be underrepresented in passive clinical surveillance.

- **Relevant for population-level transmission**

Although blood donors are a healthier subset of the population, donor-derived WNV remains informative for public health because severe disease is driven largely by host factors.

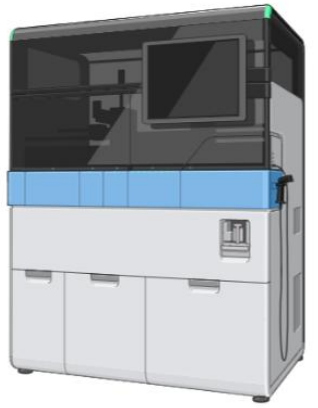
- **Scalable, systematic sampling and operationally standardized**

Blood donors represent a well-characterized, geographically distributed population that enables consistent, large-scale genomic monitoring

Limitations of donor-based WNV genomic surveillance

- **Travel confounding:** donor location may not reflect infection location; new lineages or clades could represent travel-associated infection, not local circulation in mosquitos/birds.

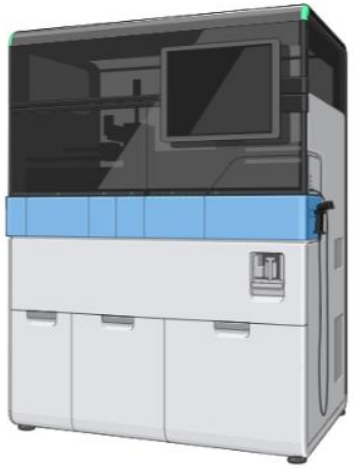
- **Detection is constrained by NAT screening performance:** donor sequencing only captures lineages and variants detected by NAT.



These limitations highlight the need for integrated genomic surveillance across donors, clinical cases, and vectors.

Study overview: sequencing workflow for NAT-reactive blood donor samples

Creative Testing Solutions



Grifols Procleix® WNV Assay

Residual NAT-reactive plasma samples
(first batch: n=134)

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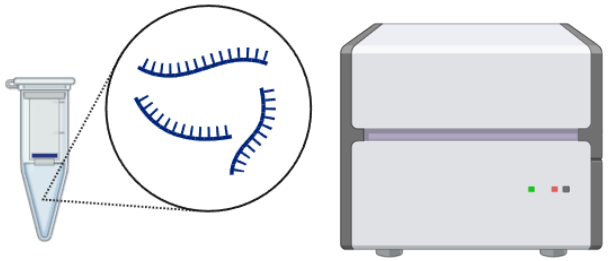
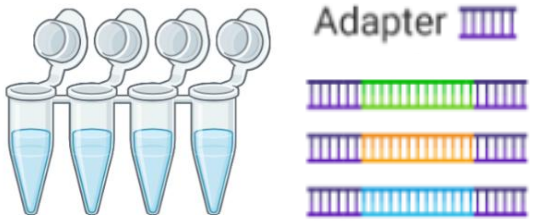


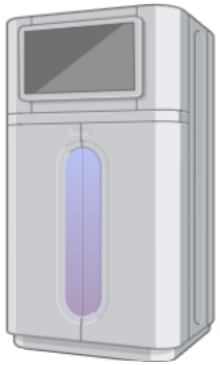
RNA extraction and RT-qPCR

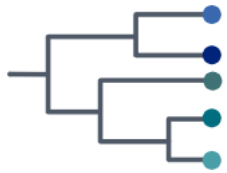
Cq used as a reference measure of viral burden to guide NGS protocol optimization



Next generation sequencing library preparation

Amplicon, metagenomic, and target-capture workflows evaluated





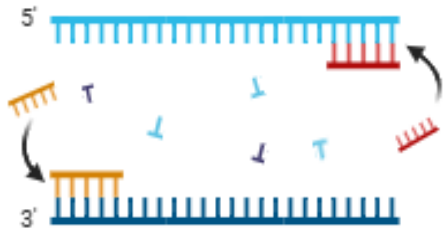
Sequencing and downstream analysis

Genome recovery, coverage, consensus, and variants

Schematic generated with BioRender

Sequencing strategy selection for low-viremia WNV donor samples

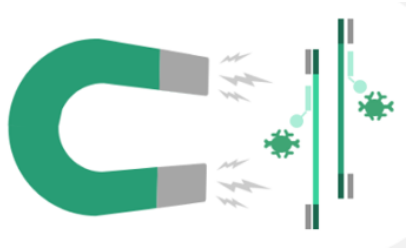
Amplicon-based sequencing (PrimalSeq)



Strength: high sensitivity, fast, cost-effective and most practical for low-viremia samples

Limitation: requires prior design typically against a specific lineage; performance declines when primer-binding sites diverge

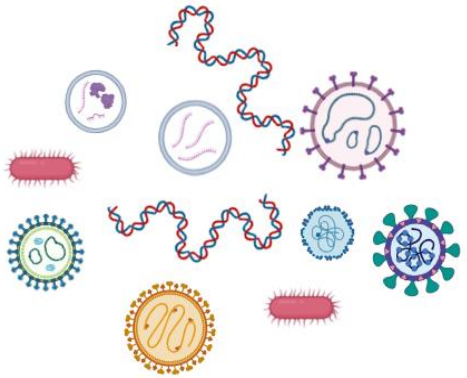
Target-capture sequencing (hybridization)



Strength: broader tolerance to sequence divergence than PCR-based approaches

Limitation: requires prior design and more complex and resource-intensive workflow

Metagenomic sequencing (mNGS)

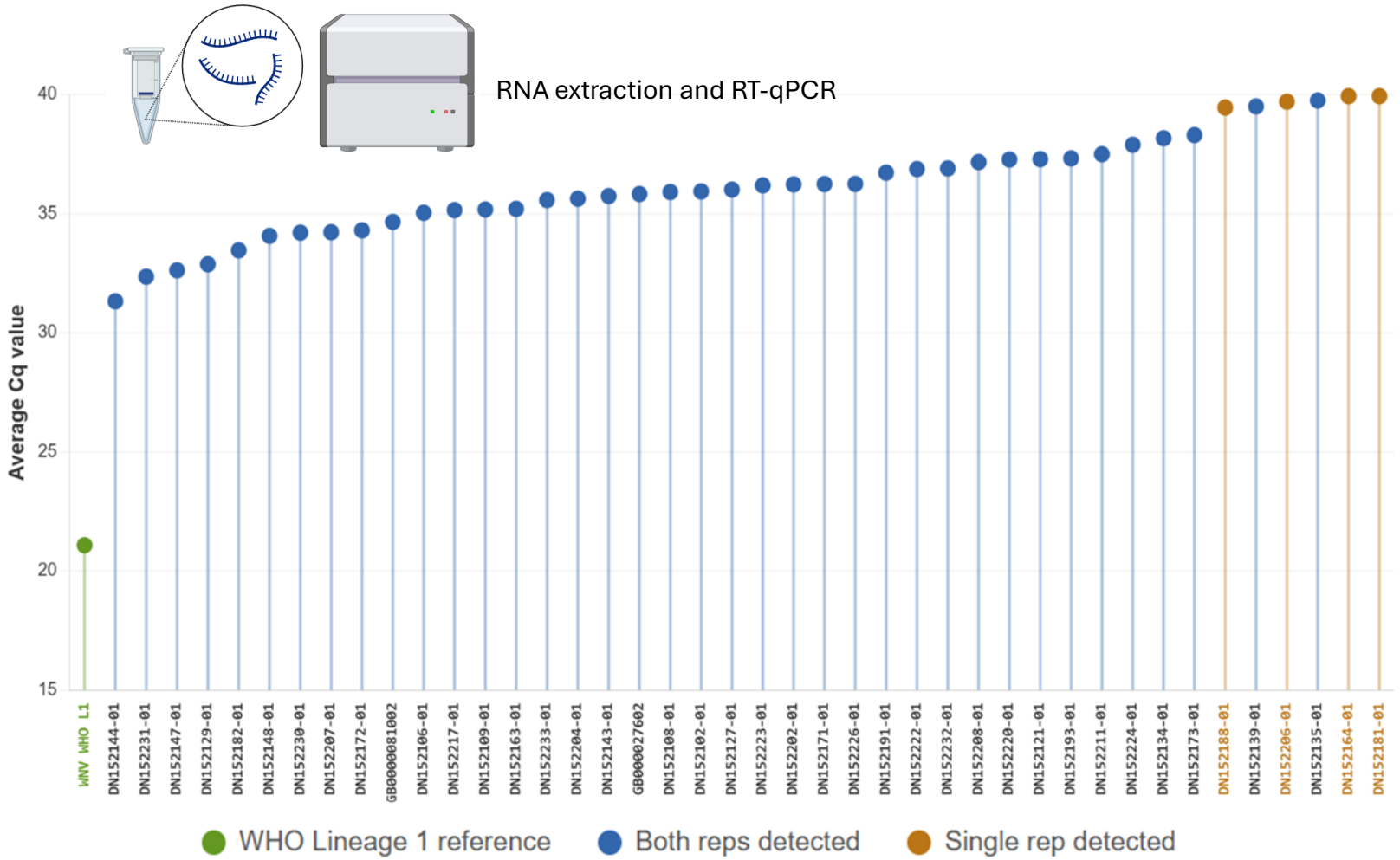


Strength: unbiased (lineage-agnostic) and capable of detecting unexpected viruses/co-infections

Limitation: less sensitive with low-viral-load samples; requires higher sequencing depth

Schematic generated with BioRender; PrimalSeq ref: Genome Biol. 2019 Jan 8;20(1):8

RT-qPCR characterization of NAT-positive donor samples

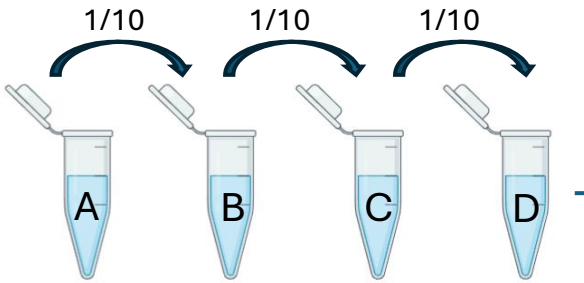


- Most NAT-reactive donor samples showed high RT-qPCR Cq values, consistent with low-level viremia
- The WHO Lineage 1 reference standard amplified at substantially lower Cq

All samples were amplified in duplicate; Cq values shown are the mean of both replicates

Cq= quantification cycle; 42 donor samples plus 1st WHO International Standard for WNV RNA (Lineage 1) (NIBSC code: 18/206)

PrimalSeq: sensitivity panel for donor-like low-viremia WNV samples

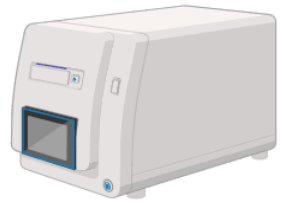


WHO L1 (Cq~30)

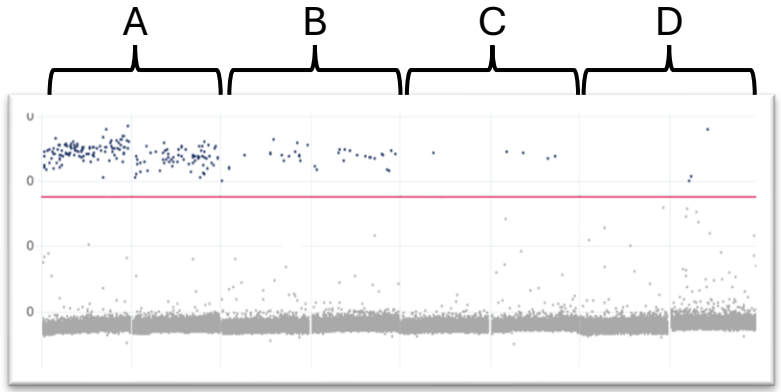
Schematic generated with BioRender

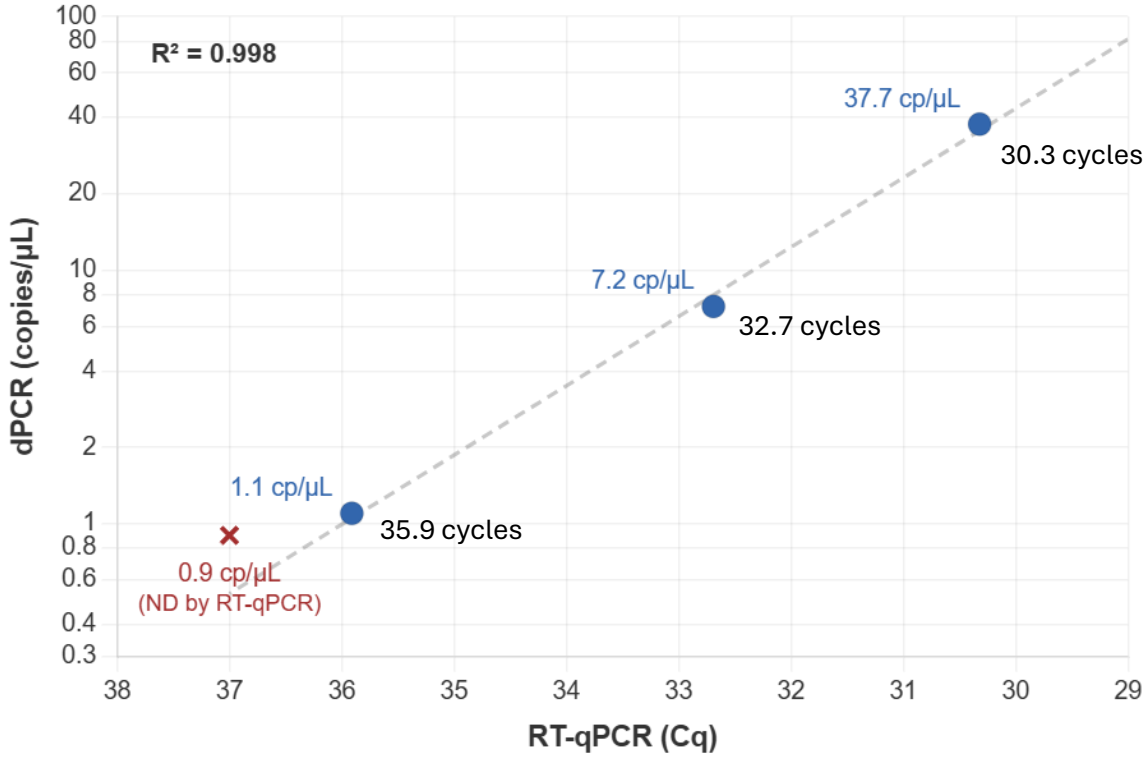
RT-qPCR

RT-dPCR



QIAcuity, 50k+ partitions

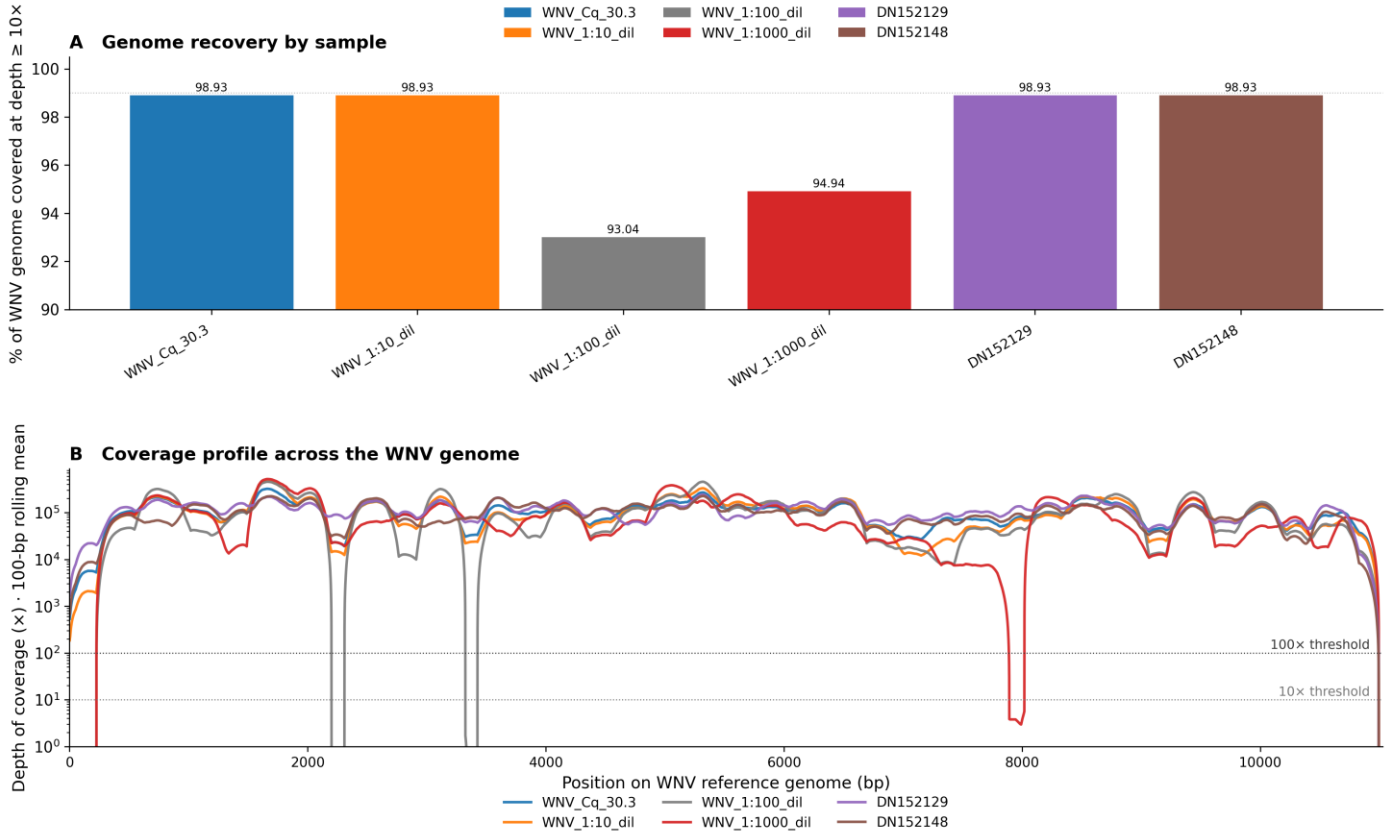




- WHO lineage 1 reference material was serially diluted 10-fold in WNV-negative plasma to span the Cq range observed in donor samples (Cq ~30-40).
- RT-dPCR provided absolute RNA input quantification for each dilution point.
- This panel enabled direct evaluation of genome recovery as a function of known viral input.

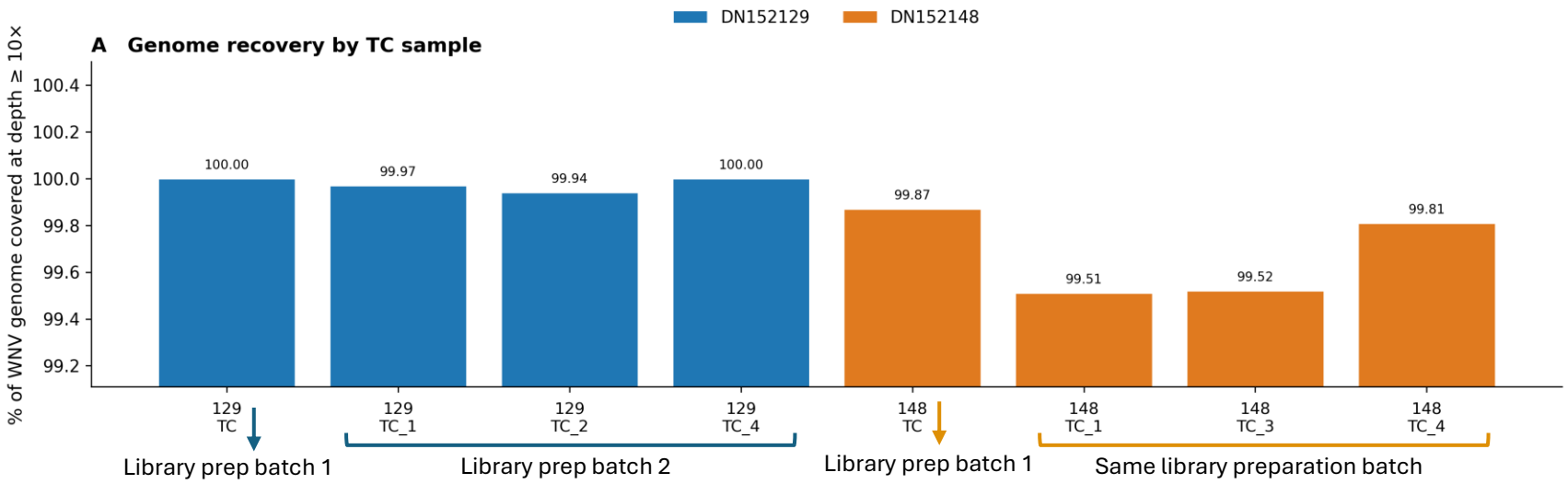
PrimalSeq assay evaluation: sensitivity panel and two blood donor samples

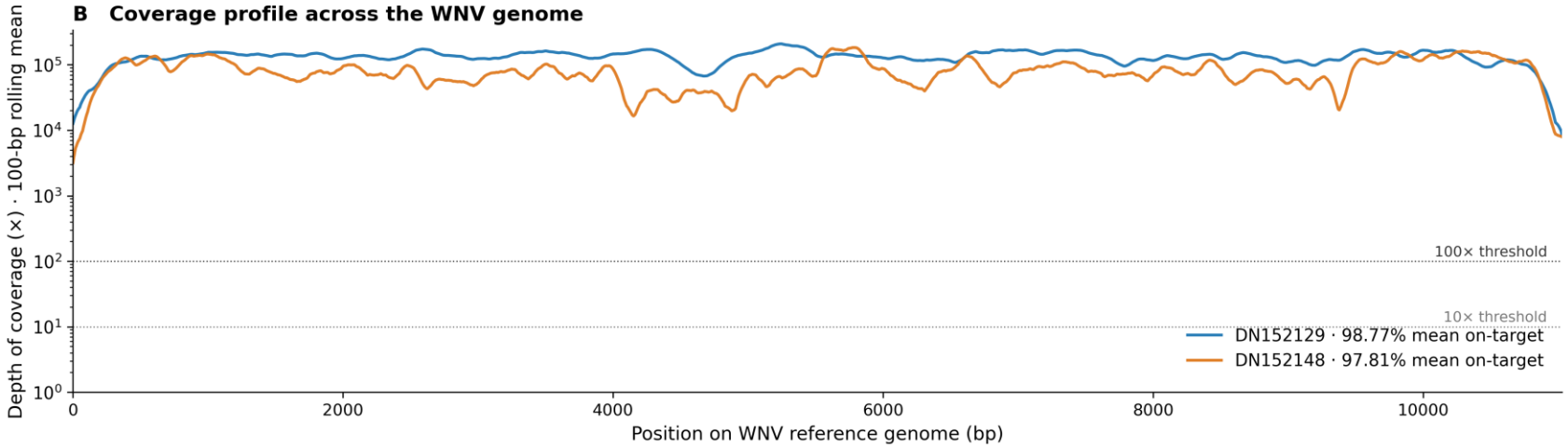
Sample ID	Mapping rate (%)	Coverage at 10x (%)	Variant count
WNV_Cq_30.3	99.9	98.9	9
WNV_1:10_dil	99.7	98.9	9
WNV_1:100_dil	98.3	93.0	9
WNV_1:1000_dil	88.1	94.9	9
DN152129	98.9	98.9	112
DN152148	99.9	98.9	111



- Across the 3-log sensitivity panel (down to ~10 copies/reaction), PrimalSeq maintained high performance, although mapping rate decreased with lower RNA input.
- Despite this reduction, genome recovery remained strong across the full dilution range and above the 70% cut-off typically required for phylogenetic analysis (Tóth et al., Journal of Infection Oct 2025).

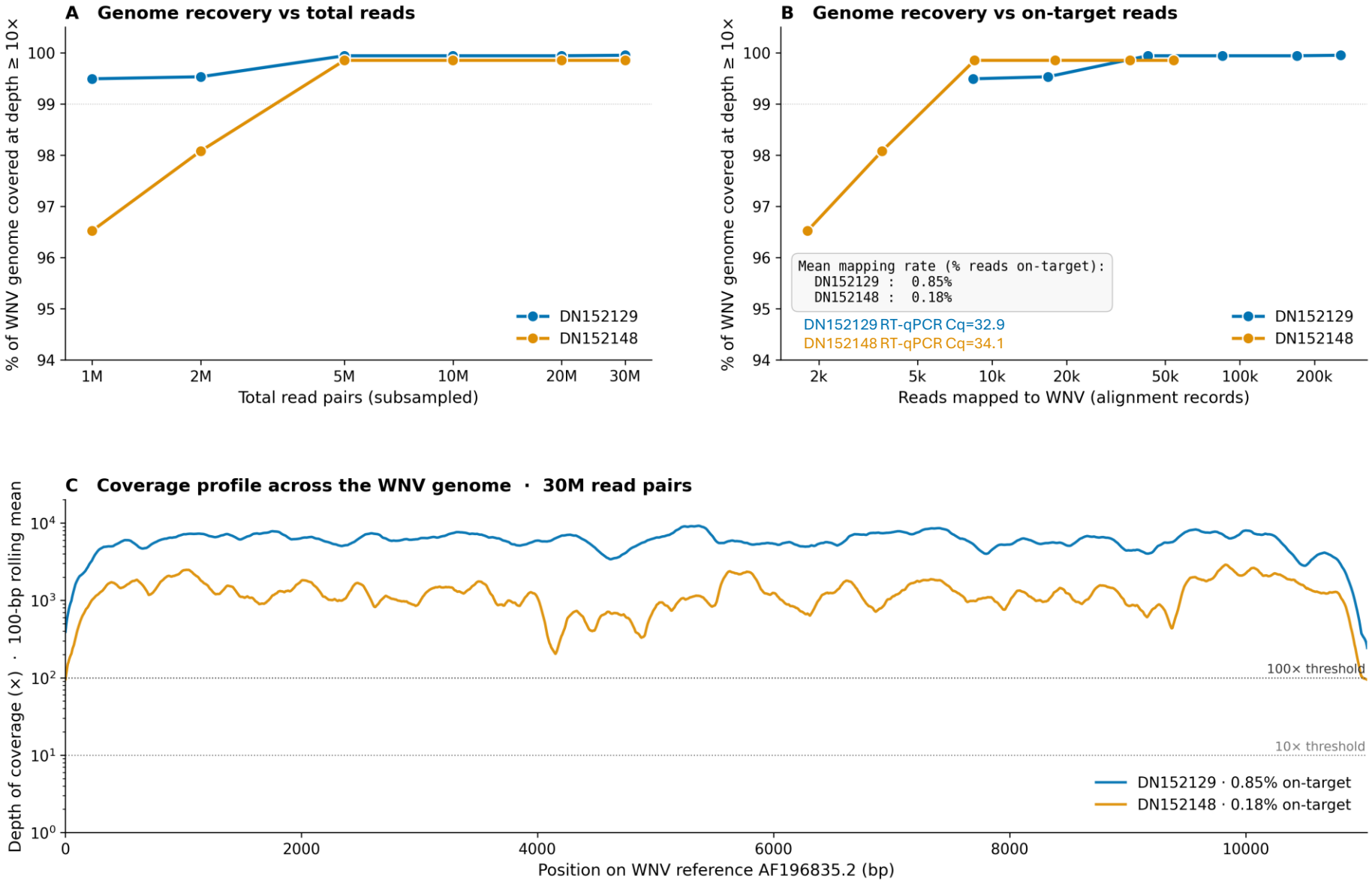
Target capture achieved high recovery and exceptional on-target enrichment





- Exceptional on-target mapping: **~98%** of reads mapped to WNV
- >**99.5%** genome recovery at ≥10× across all libraries
- Broad, uniform genome-wide coverage with no dropout
- Strong performance, but added capture steps increase workflow complexity that can limit scalability

Metagenomics (shotgun RNA sequencing) achieved complete genome recovery in donor samples

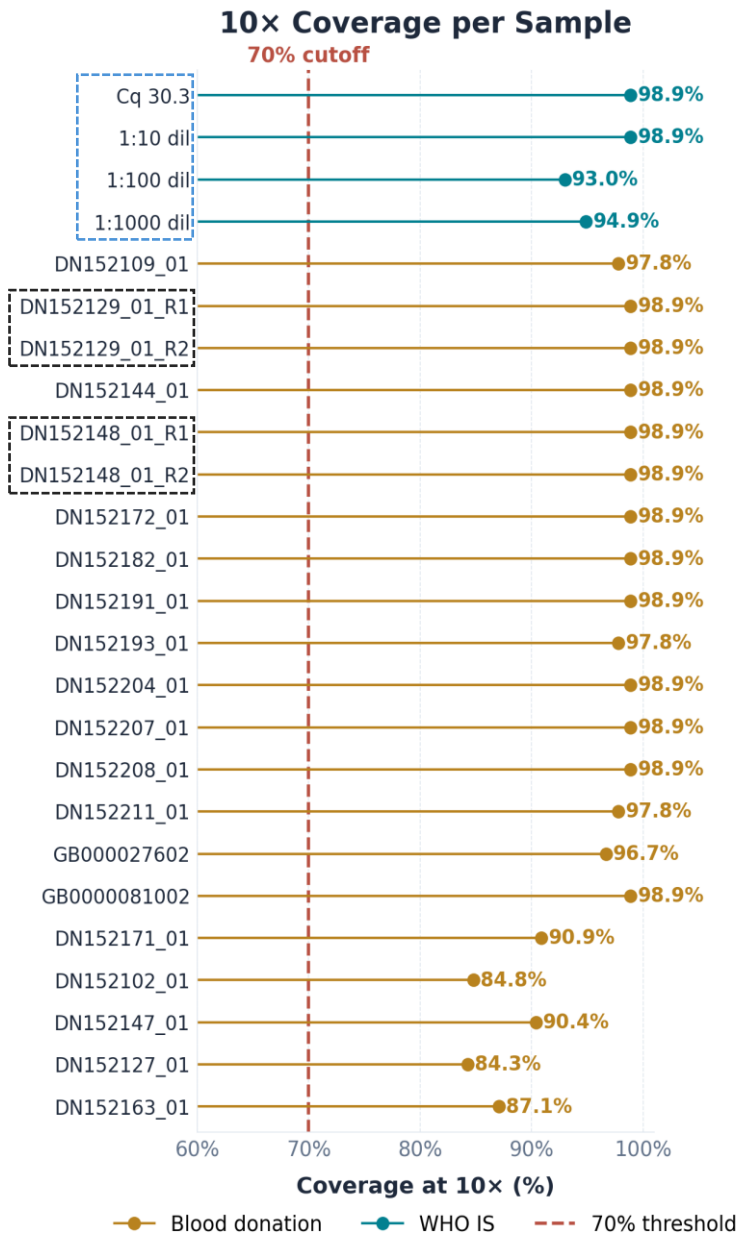


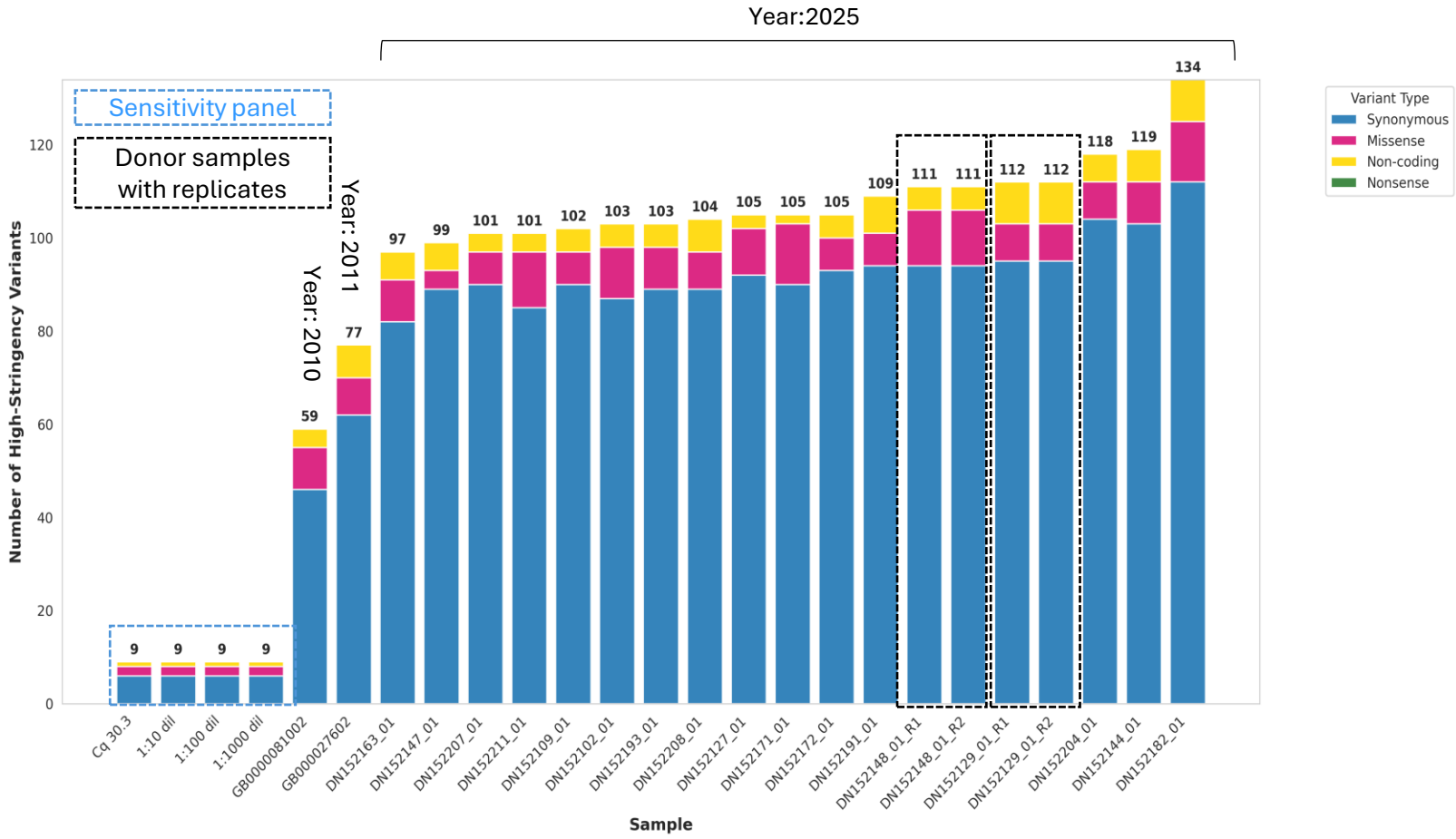
- Despite **<1% mapping rate** (expected for shotgun RNA-Seq on plasma), both samples achieved **≥99.5%** genome coverage at 10x depth by ~5M read pairs.
- Coverage remained broadly **uniform** across the genome, with expected loss only at the extreme reference ends.

From benchmarking to strategy: method selection framework

- ✓ All three approaches enabled robust consensus derivation and phylogenetic placement
- ✓ PrimalSeq is well suited as a **first-pass "scanning"** assay: rapid, sensitive, cost-effective, and informative for lineage 1a donor samples.
- ✓ PrimalSeq coverage/dropout patterns can triage samples for broader methods:
  - Target capture performed strongly, but added workflow complexity may limit routine use and scalability
  - Shotgun RNA-seq provides lineage-agnostic resolution when amplicon coverage suggests divergence, and may become **increasingly practical as sequencing costs decline**

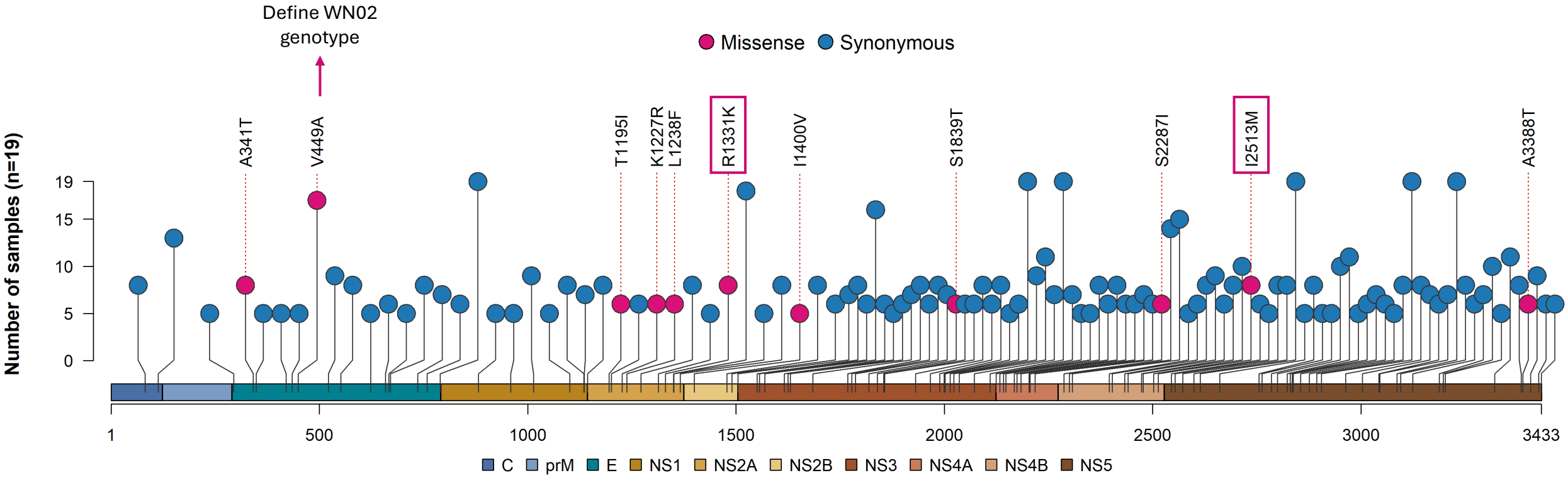
PrimalSeq coverage and variant analysis from the first donor sample set (n=19)





- Variant profiles were dominated by **synonymous** changes
- Per-donor-sample variant counts ranged from **59 to 134**, and technical replicates showed **complete concordance**, supporting assay reproducibility

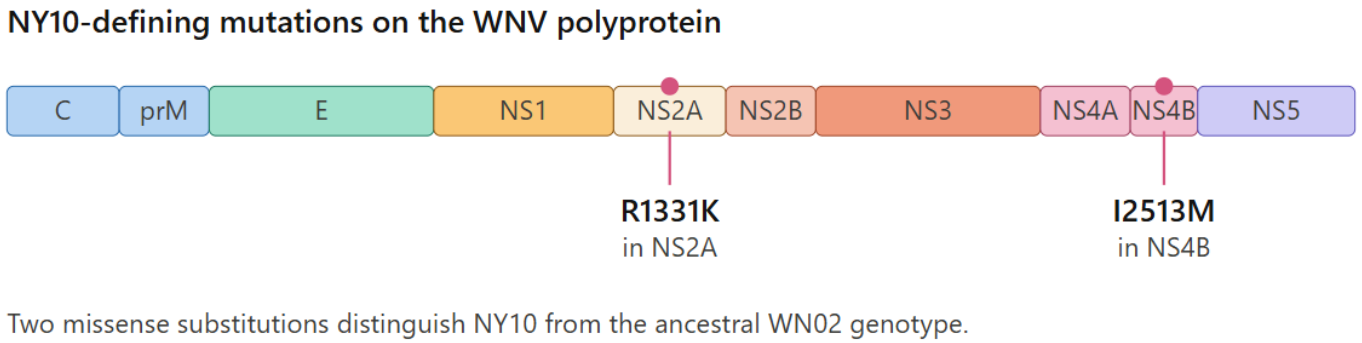
Distribution of recurrent consensus variants across the WNV polyprotein in sequenced samples



Genotype-defining mutations identified: labeled missense variants include known lineage 1a genotype markers — V449A (**WN02**), and R1331K / I2513M (**NY10**)

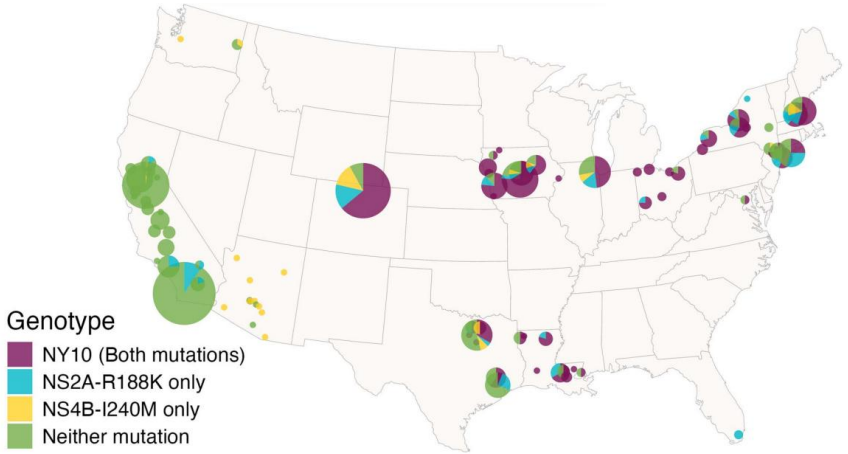
(Virus Evolution, Volume 5, Issue 2, July 2019, vez035; Emerg Microbes Infect. 2022 Dec;11(1):988-999).

West Nile Virus: Adaptive Evolution and the NY10 Genotype



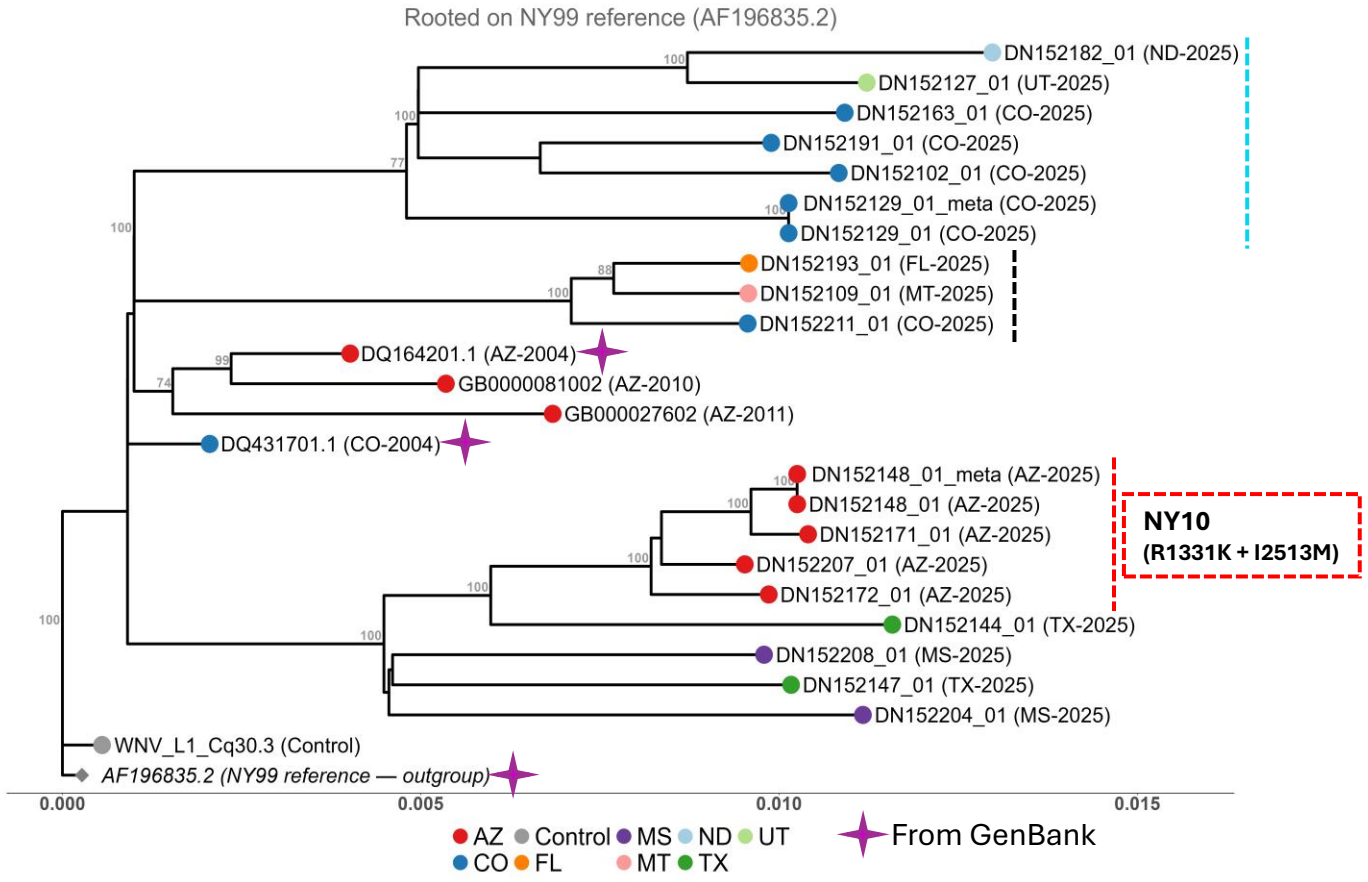
Two missense substitutions distinguish NY10 from the ancestral WN02 genotype.

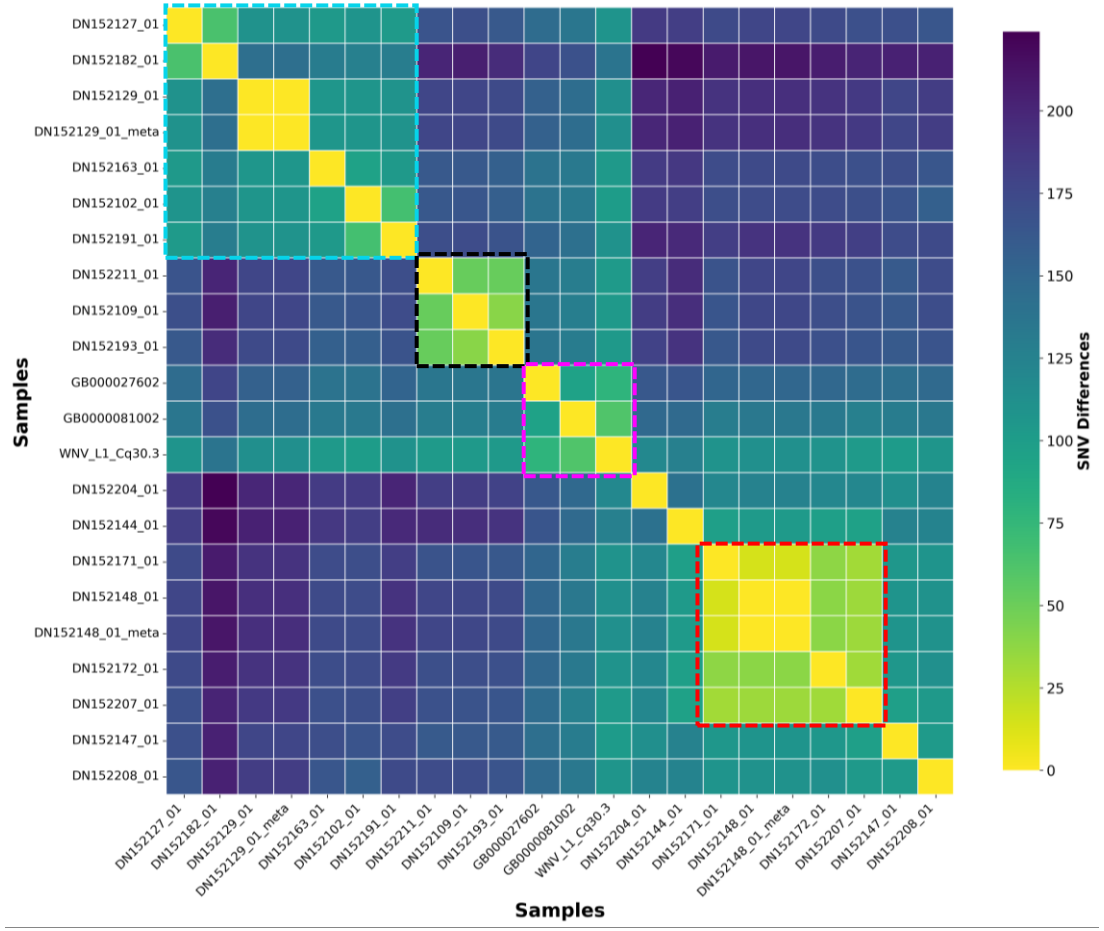
- Emerged in New York in 2010 and rapidly spread due to **fitness advantage** (Virus Evolution, Volume 5, Issue 2, July 2019, vez035; Emerg Microbes Infect. 2022 Dec;11(1):988-999).
- **2012 Outbreak Driver:** NY10 played a central role in the 2012 WNV outbreak—the largest since 2003—with the outbreak geographically restricted to eastern regions where NY10 was dominant (Virus Evolution, 2025, 11(1), veaf037)
- **Regional Adaptation:** in published datasets to date, **NY10** was shown to be geographically restricted east of the Rocky Mountains (Virus Evolution, 2025, 11(1), veaf037)



Continued genomic surveillance is essential, as WNV demonstrates capacity to generate **adaptive mutations** that significantly amplify epidemic potential on **regional scales**

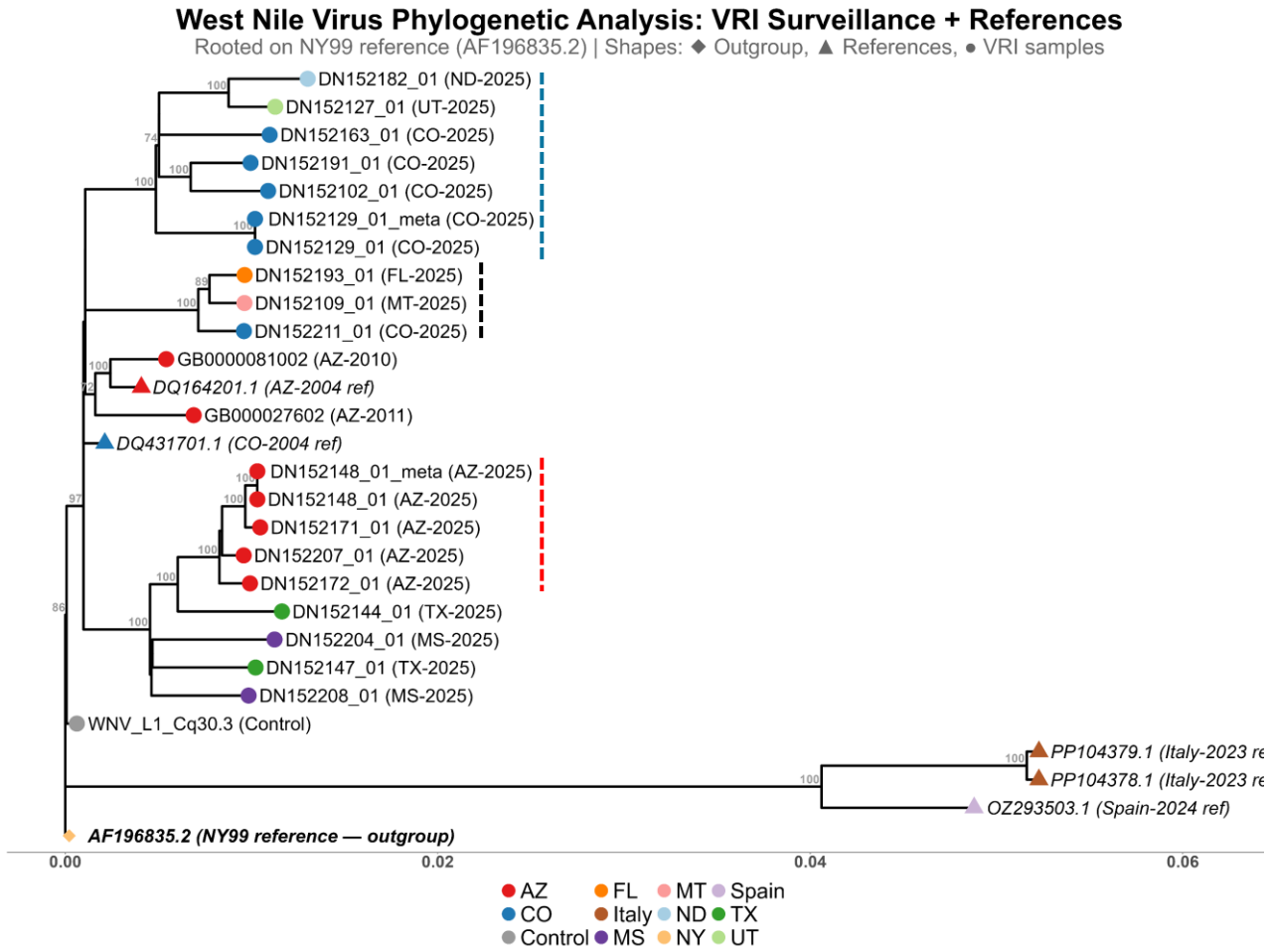
Preliminary donor-derived WNV genomes resolve temporal structure and contemporary diversity

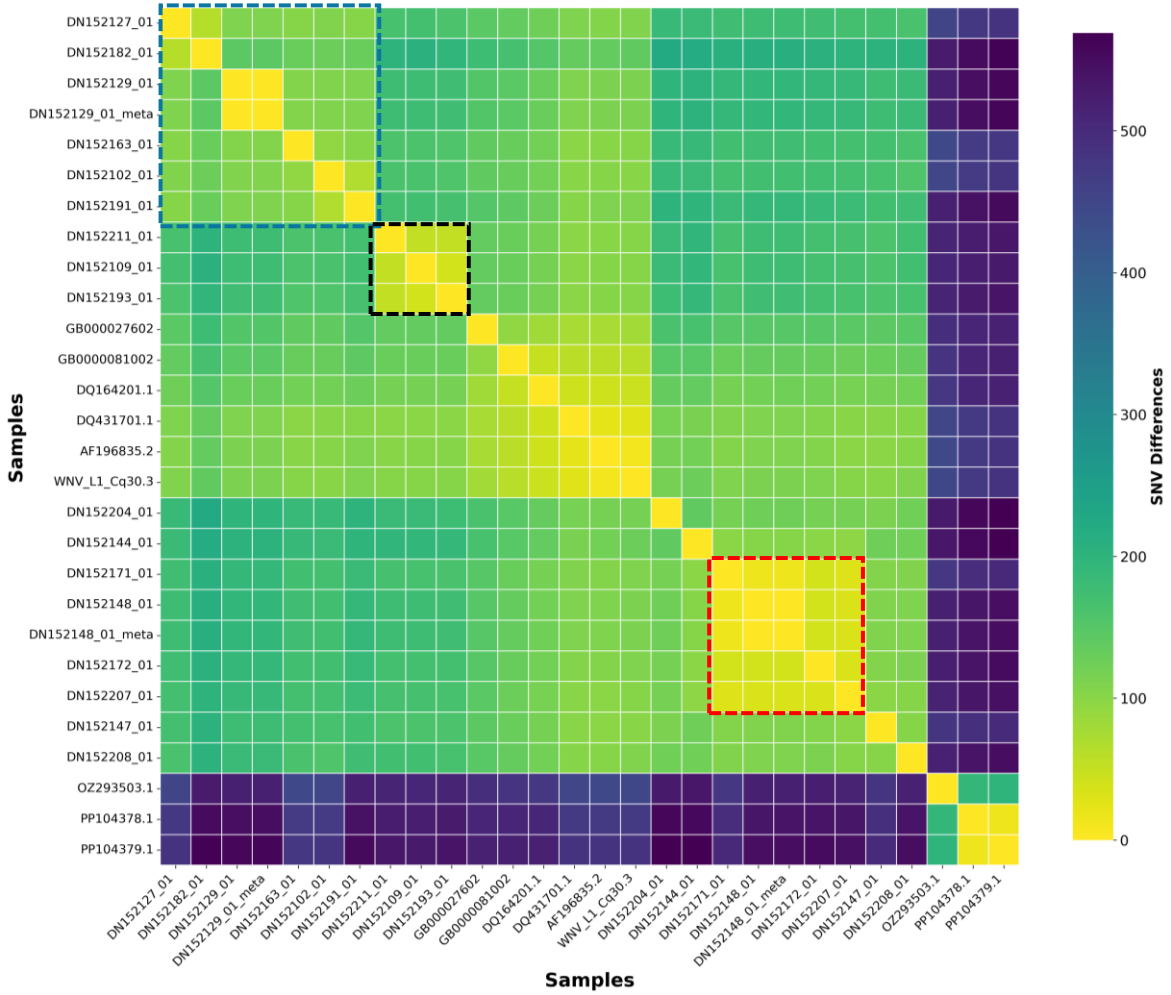




- Older 2010–2011 donor-derived genomes cluster with older GenBank reference sequences, supporting the temporal coherence of the phylogeny.
- The 2025 donor genomes resolve into multiple contemporary clusters, indicating recoverable phylogenetic diversity in current donor-derived WNV
- Cross-method genome pairs (PrimalSeq vs metagenomics, "meta") cluster tightly together, supporting technical concordance.
- In this preliminary dataset, AZ cluster is composed of **NY10 genotype**

2025 U.S. WNV genomes remain distinct from European lineage 1a sequences





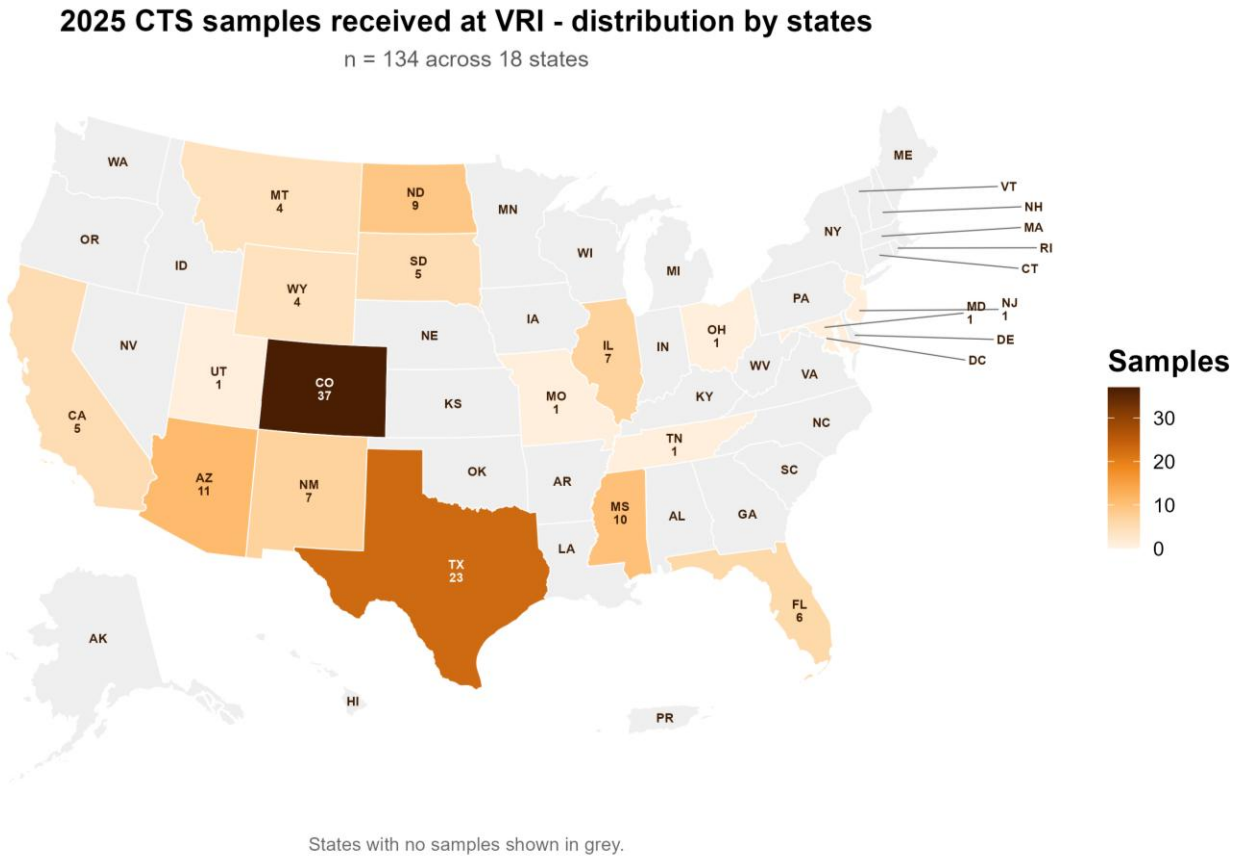
The U.S. and European lineage 1a populations are evolving largely independently. For NAT assay design, primer and probe regions may need to be monitored regionally, because what works for U.S. lineage 1a may not perform identically on European lineage 1a.

Ongoing work

- Completing sequencing of remaining 2025 CTS NAT-reactive donor samples to increase geographic coverage and evaluate spatial clustering.

- Expanding Arizona and Colorado sampling to prior transmission seasons, with emphasis on **Arizona 2021** given the large outbreak and recent evidence of NY10 detection in 2025.

- Goal: compare recent and historical donor-derived genomes to assess whether genotype patterns reflect local persistence, new introductions, or regional replacement, in association with neuroinvasive disease burden.



Donor-derived WNV genomes provide a standardized, scalable layer for integrated One Health surveillance

- Phylogeographic frameworks can link WNV lineage movement with environmental drivers such as temperature and landscape — but require dense, geographically distributed genomic data.

- Consistent NAT-reactive donor sequencing adds a standardized human-phase layer alongside mosquito, avian, and clinical surveillance — leveraging infrastructure that already exists.

Donor-derived WNV genomes may help move surveillance from retrospective outbreak description toward epidemiological hypothesis testing and, ultimately, support integrated One Health risk forecasting.

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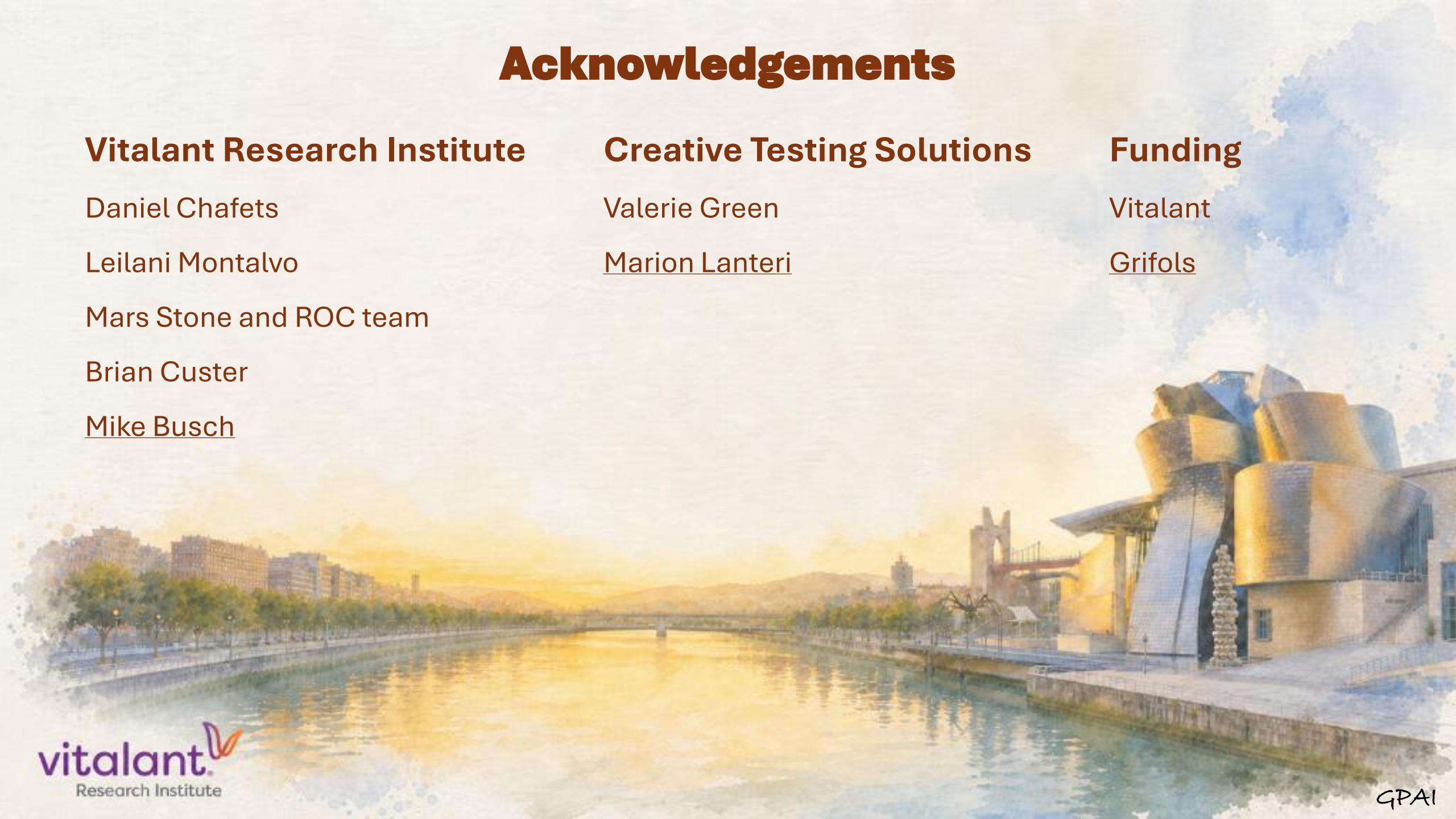

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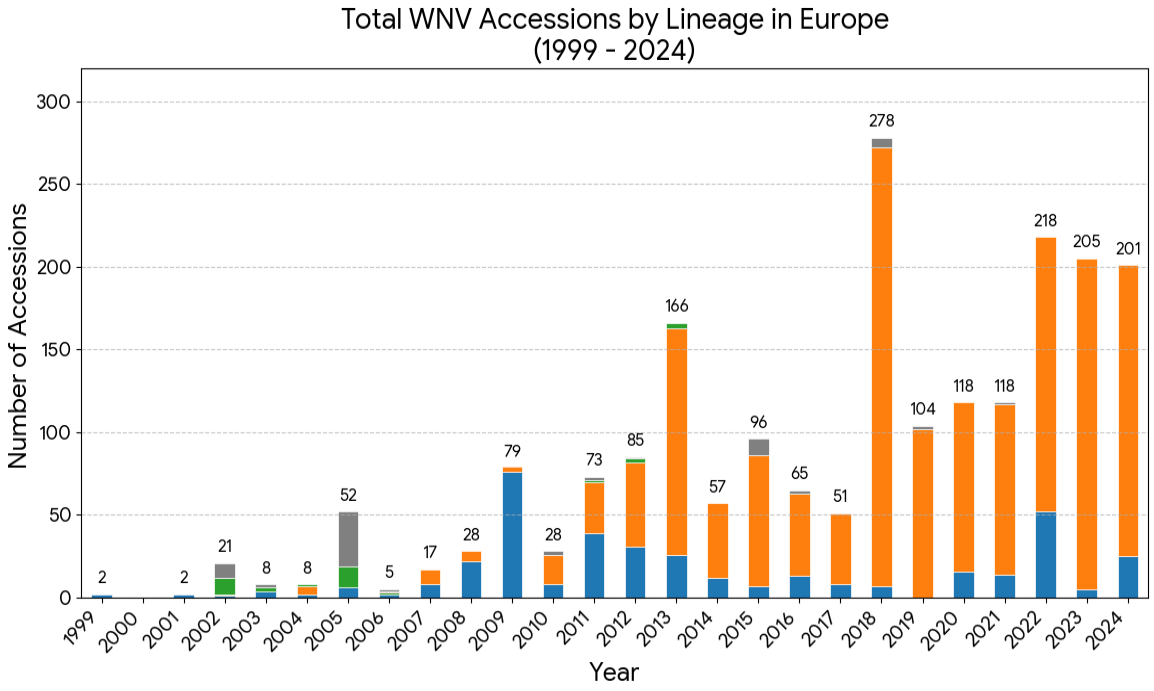
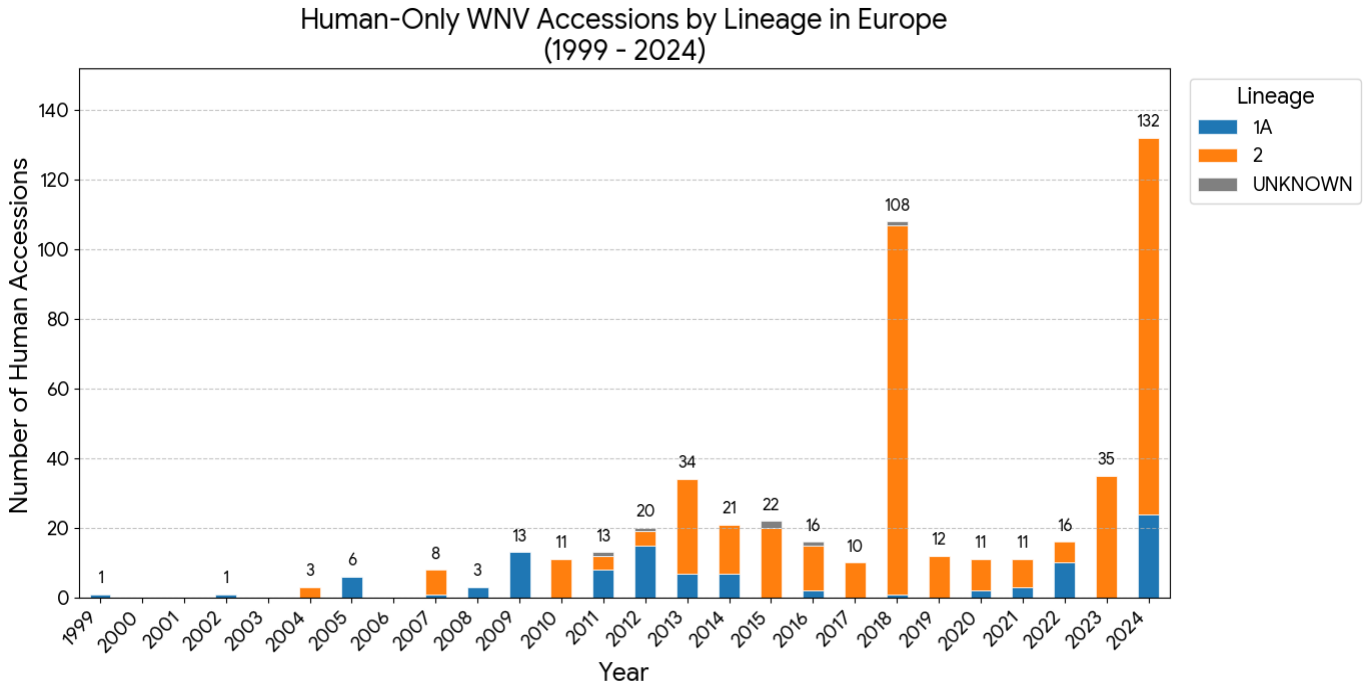
Backup slides

Europe shows recent growth in publicly available WNV genomes





Europe has recently generated more WNV genomic data, including a growing number of human-derived genomes.

Source: Publicly available WNV genomes represented in the Nextstrain WNV build (GenBank-derived metadata; accessed March 2026)

Unexpected WNV lineage diversity in a U.S. human infection highlights a surveillance gap

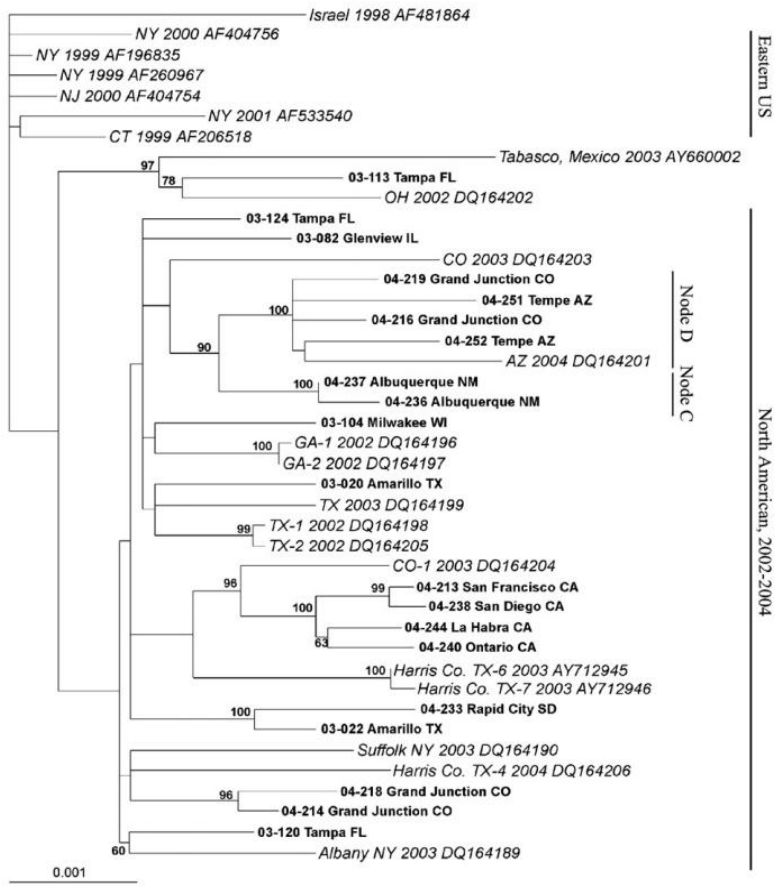
- Nebraska, August 2023: immunocompetent patient with neuroinvasive disease, ascending paralysis, and multisystem organ failure.
- Unusual high viral load prompted sequencing of serum and cerebrospinal fluid at CDC.
- Lineage 1 and **lineage 3** WNV RNA were detected in both specimen types.
- Lineage 3 had previously been identified in Central European mosquito pools and had not been documented in the U.S. before.
- L3 prevalence in US mosquito populations remains unknown.

This case suggests that WNV lineage diversity in the United States may be underrecognized without genomic surveillance.

Davis et al., *Emerg Infect Dis. 2024;30(10):2090-2098*

Historical and recent precedents: donor-derived WNV genomes

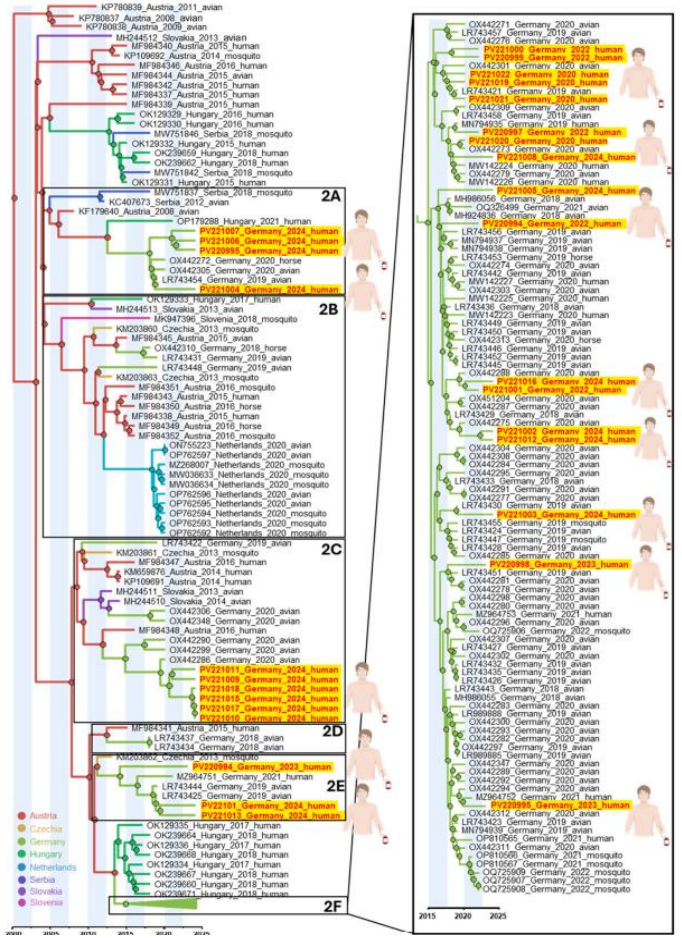
U.S. – 179 donors analyzed; 20 full ORF genomes sequenced– 2003-2004



Lineage 1a

Herring et al., Virology 2007

Germany – 43 WNV whole genomes – 2020-2024

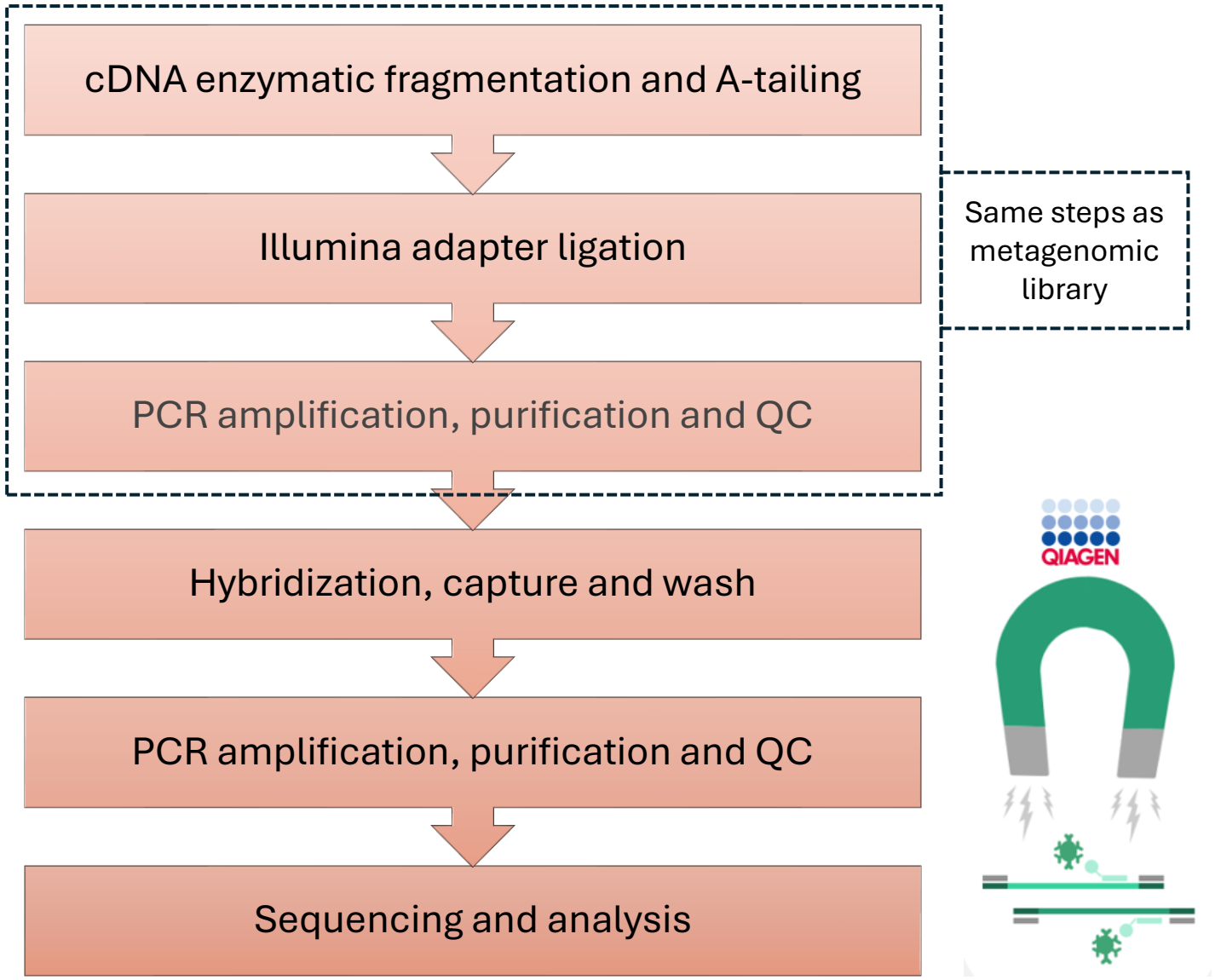


Lineage 2

Tóth et al., Journal of Infection Oct 2025

Donor-derived genomes can support real-time tracking of WNV evolution and spatial spread directly in humans.

Target capture workflow



Custom design:

- The panel covers WNV Lineage 1a, 1b, 2, 3, 4, 5; USUV; SLEV
- QIAGEN design guidance indicates that the capture probes are expected to tolerate up to ~10% sequence mismatch without substantial loss of performance.
- Designed to: reduce lineage bias; enable detection of unexpected introductions; support future collaborations across different geographic regions (e.g., Europe)

Metagenomic (shotgun RNA sequencing) workflow: improving viral signal by host DNA/rRNA depletion

DNase I digestion to remove host DNA from plasma-extracted RNA

Reverse transcription with rRNA and globin RNA removal

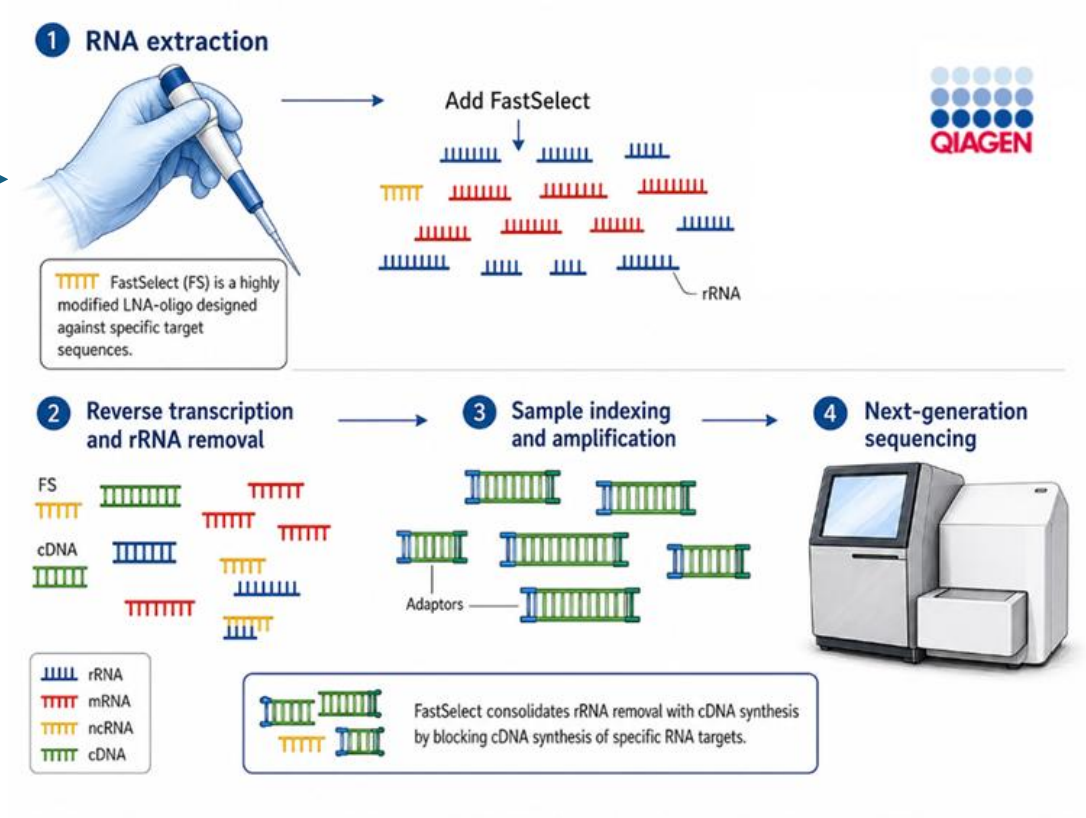
cDNA enzymatic fragmentation and A-tailing

Illumina adapter ligation

PCR amplification

Library purification and QC

Sequencing and analysis



Modified from: https://www.nature.com/articles/d42473-023-00068-x