Whole genome amplification of West Nile virus lineage 2

Anna Nagy[1]

[1]National Reference Laboratory for Viral Zoonoses, National Public Health Center

National Public Health Center, Hungary

Anna Nagy
National Reference Laboratory for Viral Zoonoses, National P...

JAN 30, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.e6nvwjopdlmk/v1

Protocol Citation: Anna Nagy 2023. Whole genome amplification of West Nile virus lineage 2. **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvwjopdlmk/v1

Protocol status: Working
We use this protocol and it's working

Created: Jan 29, 2023

Last Modified: Jan 30, 2023

PROTOCOL integer ID: 76038

Keywords: West Nile virus, lineage 2, Whole genome amplification, one-step RT-PCR

ABSTRACT

West Nile virus is one of the most important endemic arbovirus in Hungary. For next-generation whole genome sequencing of West Nile virus lineage 2, a one-step reverse transcription PCR assay was developed. PCR amplicons can be used for further amplicon based sequencing protocols on Illumina MiSeq platform. The genome of the West Nile virus is a single-stranded, positive-sense, capped RNA of approximately 10–11 kb in length. The whole genome amplification was carried out by twelve overlapping PCR amplicons. For ampification of the whole genome primer sets were designed by Geneious Prime (version 2021.2.2) primer design tool.

Nucleic acid extraction

1 Use ***Qiagen QIAamp Viral RNA Mini Kit*** (cat. no. 52904 or 52906) for nucleic acid extraction. Nucleic acid extraction should be done by following the manufacturer's instructions. The total volume of the extracted viral RNA is 60 µl.

One-Step Reverse transcription (RT) PCR Setup

2 Reagent name: ***Invitrogen™ SuperScript™ III One-Step RT-PCR System with Platinum™TaqHigh Fidelity DNA Polymerase*** (cat. no. 12574030 or 12574035)
Keep all components, reaction mixes, and samples on ice. After preparation of the samples, transfer them to the preheated thermal cycler and immediately start the RT–PCR program. West Nile virus (WNV) whole genome can be amplified by 12 overlapping fragments for each sample. Detailed description of primer sets for the whole genome amplification of WNV lineage 2 can be found in the Word file (Primers_WNV_whole_genome_amplification) attached below.

Primers_WNV_whole_genome_amplification.docx

PCR setup:

Add the following to a 0.2–mL, nuclease-free, thin-walled PCR tube on ice:

	A	B
	Components	Volume (µl) for 1 x rxn
	SuperScript™ III RT/ Platinum™ Taq High Fidelity Enzyme Mix	0.5
	2X Reaction Mix (a buffer containing 0.4 mM of each dNTP, 2.4 mM MgSO4)	12.5
	Autoclaved distilled water	5.0
	Sense primer (10 µM)	1.0
	Anti-sense primer (10 µM)	1.0
	Total volume	20.0
	Template RNA (1 pg to 1 µg)	5.0

	A	B
	Final volume	25.0

PCR master mix components for 25.0 µl reactions.

For multiple reactions, you can prepare a master mix to minimize reagent loss and enable accurate pipetting.

3 Program the thermal cycler so that cDNA synthesis is followed immediately with PCR amplification automatically:

	A	B	C	D
	Reverse transcription	55°C	30 mins	1x
	Activation/initial denaturation	94°C	2 mins	1x
	Amplification	94°C	15 sec	40x
		59°C	30 sec	
		68°C	4 mins 30 sec	
	Final extension	68°C	5 mins	1x
	HOLD	12°C	infinite	infinite

Cycling conditions for whole genome amplification of West Nile virus lineage 2

Gently mix and make sure that all the components are at the bottom of the amplification tube. Centrifuge briefly if needed. Place the reaction tubes in the preheated thermal cycler programmed as described above.

Agarose gel electrophoresis

4 For visualization and interpretation of the results 1% agarose gel should be used.