Nov 28, 2022

Whole genome amplification of dengue virus type 1 - 4

DOI
dx.doi.org/10.17504/protocols.io.6qpvr486pgmk/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.6qpvr486pgmk/v1

Protocol Citation: Anna Nagy 2022. Whole genome amplification of dengue virus type 1 - 4. **protocols.io https://dx.doi.org/10.17504/protocols.io.6qpvr486pgmk/v1**

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

Created: November 26, 2022

Last Modified: November 28, 2022

Protocol Integer ID: 73258

Keywords: whole genome amplification of dengue virus type, generation whole genome sequencing of dengue virus, genome of the dengue virus, dengue virus type, dengue virus, whole genome amplification, reverse transcription pcr assay, pcr amplicon, step reverse transcription pcr assay, whole genome sequencing, overlapping pcr amplicon, imported tropical viral infection, tropical viral infection in hungary, rna, genome, capped rna, sequencing protocol, primer design tool, different primer set, pcr, nucleotide in length, geneious prime, nucleotide

Abstract

Dengue virus is one of the most often imported tropical viral infection in Hungary. For next-generation whole genome sequencing of dengue virus serotypes 1 - 4, 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 dengue virus is a single-stranded, positive-sense, capped RNA of approximately 10–11 kb in length. The whole genome amplification was carried out by five overlapping PCR amplicons, each one is approximately 2500 nucleotide in length. For ampification of the four serotypes, four different primer sets were designed by Geneious Prime (version 2021.2.2) primer design tool.

Troubleshooting

1 Nucleic acid extraction:
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.

2 One-Step Reverse transcription (RT) PCR Setup

2.1 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. Dengue virus whole genome can be amplified by 5 overlapping fragments for each sample. Detailed description of primer sets for the whole genome amplification of DENV serotypes 1 - 4 can be found in the Excel sheet (Primers_DENV_whole_genome_amplification) attached below.

Primers_DENV_whole_genome_am...

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

	A	B
	Anti-sense primer (10 µM)	1.0
	Total volume	20.0
	Template RNA (1 pg to 1 µg)	5.0
	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.

2.2 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
		53°C	30 sec	
		68°C	4 mins 30 sec	
	Final extension	68°C	5 mins	1x
	HOLD	12°C	infinite	

Cycling conditions for whole genome amplification of dengue virus type 1 - 4

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.

3 Agarose gel electrophoresis: for visualization and interpretation of the results 1% agarose gel should be used.