Title: Reverse Transcription (RT) for First-strand cDNA synthesis		
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Reverse Transcription (RT) for First-strand cDNA synthesis

I. PURPOSE

To reverse transcribe total RNA and synthesize first-strand cDNA for downstream Next-Generation Sequencing library preparation.

II. MATERIALS & EQUIPMENTS

Sample type

- Extracted arboviral RNA

Reagents

- SuperScript® III First-Strand Synthesis System for RT (Invitrogen Cat# 18080051)
- Nuclease – free ultrapure water

Consumables

- 0.2ml PCR tubes

Equipment

- Thermal cycler

III. PROCEDURE

Note: Use 8ul (maximum amount) of extracted RNA and 1ul of random hexamers for this protocol with Dengue and Zika virus.

1. Prepare RNA mixture:
   a) Prepare RNA mixture with the following components in a 0.2 ml PCR tube

Component	Amount/ sample
Extracted Sample RNA	*8* uL
Primers - 50 ng/µL random hexamers	1 uL
10 mM dNTP mix	1 uL
Nuclease-free water *	0 ul
Total volume	*10 ul*

* top up to 10 ul water if sample is less than 8 ul

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b) Place tube in thermal cycler at 65°C for 5 mins
c) Then incubate on ice for 1 min and quick spin down.

- Prepare the cDNA synthesis mix.
  a) In a separate tube, add each component in the following indicated order:

Component	**1 Rxn**	**10 Rxns**
10X RT buffer	2 uL	20 uL
25 mM MgCl2	4 uL	40 uL
0.1 M DTT	2 uL	20 uL
RNaseOUT™ (40 U/µL)	1 uL	10 uL
SuperScript® III RT (200 U/µL)	1 uL	10 uL
Total volume	*10 ul*	*100 ul*

b) Mix well by gently pipetting up and down. Quick spin down.

- Pipette 10ul of cDNA synthesis mix to each tube with sample RNA mixture. Gently pipet to mix and quick spin.
- Place PCR tubes in thermocycler and incubate as follows:
  a. Random hexamer primed: 25°C for 10 mins, followed by 50°C for 50 mins
- Inactivate the reaction by heating to 85°C for 5 mins. Then chill on ice.

Note: The cDNA can now be used as a template for amplification in PCR. However, amplification of some PCR targets (those > 1 kb) may require the removal of RNA complementary to the cDNA.

- To remove RNA complementary to cDNA, add 1 ul (2units) of *E. coli* RNase H.
- Incubate at 37°C for 20 mins.
- cDNA synthesis reaction can be stored at -20°C or used for PCR immediately.