Title: PCR amplification of Dengue and Zika virus cDNA		
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PCR amplification of Dengue and Zika virus cDNA

I. PURPOSE

To amplify dengue virus and zika virus cDNA for downstream Next-Generation Sequencing library preparation.

II. MATERIALS & EQUIPMENTS

Samples

- Dengue or zika cDNA

Reagents

- Platinum™ Taq DNA Polymerase (Invitrogen Cat # 10966018)
- Mag-Bind TotalPure NGS magnetic beads (SciMed Asia Pte Ltd, M1378-01)
- Arbovirus-specific Forward and Reverse Primers
  - Dengue virus serotype-specific primers from Nathan protocol
  - Zika virus-specific primers from Quick protocol.
- Nuclease – free ultrapure water
- Absolute (100%) Ethanol
  - Freshly prepared 80% Ethanol in nuclease – free water from 100% Ethanol
- Agarose powder (1st base, BIO-1000-500g)
- 50X TAE buffer (1st Base, BUF-3000-50x4L)
- Gel Red dye (Merck, SCT122)
- 100bp DNA ladder
- Qubit 1X dsDNA HS kit (ThermoFisher Scientific, Q33231)

Consumables

- 0.2ml PCR tubes
- 1.5ml Eppendorf DNA Lo-Bind tubes
- Qubit 0.5ml assay tubes (ThermoFisher Scientific, Q32856)

Equipment

- Thermal cycler
- Gel electrophoresis apparatus (gel casting tray, tank and power pack)
- DynaMag-2 Magnetic Stand (Invitrogen, 12321D)
- Qubit 4 Fluorometer (ThermoFisher, Q33238)

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III. PROCEDURE

Step 1

PCR amplification:

1. Thaw all reagent components at room temperature, flick mix and quick spin down. Place on ice.

2. Prepare the PCR Master Mix
   a. Add the following components in a 0.2 ml PCR tube:

Component	**50 ul reaction**	**Final conc.**
Nuclease-free water	38.3 ul	-
10X PCR Buffer	5 ul	1X
50 mM $MgCl_2$	1.5 ul	1.5 mM
10 mM dNTP mix	1 ul	0.2 mM each
Platinum™ *Taq* DNA Polymerase	0.2 ul	2 U/rxn

   b. Pipette mix and briefly spin down components

3. Transcribed cDNA and primers
   a. Add template DNA and primers to each tube.

Note: For each tube, PCR master Mix plus template DNA and primers should total 50ul.

Component	**50 ul reaction**	**Final conc.**
10 uM Primer Pool 1 or Pool 2	2 ul	0.4 uM
Transcribed sample cDNA	2 ul	-

   b. Mix well and spin down

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4. Incubate tubes in a thermal cycler with the following program:

Lid temp. = 100°C, Reaction volume = 50ul			
1	Initial denaturation	98°C	3 mins
2 (35 cycles)	Denature	98°C	15 secs
	Anneal & Extend	63°C	5 mins
3	Hold	20°C	Indefinitely

5. Analyse the samples using gel electrophoresis
6. Proceed with magnetic beads clean-up as below

Step 2

Magnetic beads clean-up:

1. Thaw magnetics beads at room temperature for at least 30 mins before use. Vortex beads for at least 1 minute to ensure that they are fully resuspended.
2. Combine PCR reactions of Pool 1 and Pool 2 in a new 1.5 ml tube.
3. Add **90 µl** of magnetic beads (0.9x ratio) to **100 µl** PCR reaction mix and mix well by pipetting 6-10 times.
4. Incubate the beads mixture at room temperature for 5 mins.
5. Place the tubes on the magnetic stand.
6. When the solution is clear, discard the supernatant. Wash the beads by adding 1000 µl of 80% ethanol to each tube for approximately 30 seconds, taking care not to disturb the pellet.

   Note: 80% ethanol should be made fresh.
7. Discard the supernatant.
8. Repeat steps 6 and 7 once. Aspirate as much supernatant from the tube as possible and let the beads dry until matte but not cracked.

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9. Add **42 µl** nuclease-free water to each tube and incubate for at least 2 minutes at room temperature.
10. Place the tubes on the magnetic stand.
11. When the solution has cleared, transfer **40 µl** of the eluate to a new set of tubes.
12. Quantify samples using Qubit (eg, Qubit 1x dsDNA HS kit).
13. Store at -20°C or use immediately in downstream processes such as NGS library preparation.