Protocol 4E: YFV: 500 bp scheme PCR (total 54 primers, so 28 in pool A and 26 in pool B) Appropriate for all 3 designs

Make sure the primers pools are diluted to total 10uM of primers from the 100uM stock.

In total, the final concentration of primers should be 0.015uM per primer. For pool A 500bp scheme, this means 1.05ul of primers needed. For pool B, this means 0.975ul of primers is needed. Use 1ul of each of simplicity.

Choose the correct primer pool of YFV primers based on your location – e.g., Angola should use East Africa YFV primers, and Brazil should use SA1 YFV primers.

Set up two PCR mastermixes with the following components, with enough for the number of reactions + approx. 10%. In one tube, add primers for primer pool A, in the other tube, add primers for primer pool B.

Component	**Amount (ul)**	**Final concentration**
PCR grade water	15.75	
Q5 reaction buffer (5x)	5	
dNTPs (10mM)	0.5	
Primers for the pool (forward and reverse) 10uM	1	0.015 uM per primer
Q5 DNA polymerase	0.25	

Pipette out 22.5ul into PCR tubes, and add 2.5ul of cDNA to each tube**. Include a negative control.**

Run on a thermocycler at:

Cycle numbers	Step	Temperature (C)	Time
1	Initial denature	98	1 minute
35**	Denature	98	30 seconds
	Anneal and extend	65	5 minutes
1	Hold	5	Infinity

**NB, this can be adapted depending on the CT values and concentrations we achieve.

Perform PCR cleanup and Qubit for concentrations (optionally run on 1-2% agarose gel to check band size) (EGel 2%).

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ZIKA IN BRAZIL REAL TIME ANALYSIS

PROTOCOL FOR DENV-2 980bp SCHEME SEQUENCING PCR

This is a protocol for DENV-2 multiplex PCR reactions prior to the library preparation of the Oxford Nanopore sequencing. Reaction standardization and primers design were performed by PhD Sarah Hill, University of Oxford.

Materials

- DENV-2 primers pool A and B
- Micropipettes and tips
- Microcentrifuge tubes size 1,5mL
- PCR microtubes with attached caps size 0.2 mL
- Disposable lab coats
- Disposable powder-free lab gloves
- 0,2mL microtube racks
- Thermocycler

Mastermix

All primers pools should be diluted to a total of 10uM from the 100uM stock.

Components	***Amount (uL)***
Nuclease Free water	16,26
Q5 reaction buffer (5x)	5
dNTPs (10mM)	0,5
Primers for the pool 10uM	0,49
Q5 DNA polymerase	0,25

Prepare 2 mastermixes with the above components for the appropriate number of samples. In one tube add the primers of the pool A and in the other tube, add primers of the pool B.

After the mastermix is finished, pipette out 22.5uL of the mastermix into PCR microtubes, and then add in each tube 2.5ul of the appropriate cDNA.

Thermocycling parameters

Step	***Temperature***	***Time***	***Cycle number***
Initial denature	98ºC	1 minute	1
Denature	98ºC	30 seconds	35
Annealing and extension	65ºC	5 minutes	
Hold	4ºC	∞	1

ZIKA IN BRAZIL REAL TIME ANALYSIS

PROTOCOL FOR DENV-1,3,4 800-900bp SCHEME SEQUENCING PCR

This is a protocol for DENV-4 multiplex PCR reactions prior to the library preparation of the Oxford Nanopore sequencing. Reaction standardization and primers design were performed by PhD Sarah Hill, University of Oxford.

Materials

- DENV-1, 3 or 4 primers pool A and B
- Micropipettes and tips
- Microcentrifuge tubes size 1,5mL
- PCR microtubes with attached caps size 0.2 mL
- Disposable lab coats
- Disposable powder-free lab gloves
- 0,2mL microtube racks
- Thermocycler

Mastermix

All primers pools should be diluted to a total of 10uM from the 100uM stock.

Components	***Amount (uL)***
Nuclease Free water	16,11
Q5 reaction buffer (5x)	5
dNTPs (10mM)	0,5
Primers for the pool 10uM	0,64
Q5 DNA polymerase	0,25

Prepare 2 mastermixes with the above components for the appropriate number of samples. In one tube add the primers of the pool A and in the other tube, add primers of the pool B.

After the mastermix is finished, pipette out 22.5uL of the mastermix into PCR microtubes, and then add in each tube 2.5ul of the appropriate cDNA.

Thermocycling parameters

Step	***Temperature***	***Time***	***Cycle number***
Initial denature	98ºC	1 minute	1
Denature	98ºC	30 seconds	35
Annealing and extension	65ºC	5 minutes	
Hold	4ºC	∞	1

ZIKA IN BRAZIL REAL TIME ANALYSIS

PROTOCOL FOR ZIKV SEQUENCING PCR

This is a protocol for ZIKV multiplex PCR reactions prior to the library preparation of the Oxford Nanopore sequencing.

Materials

- ZIKV primers pool A and B
- Micropipettes and tips
- Microcentrifuge tubes size 1,5mL
- PCR microtubes with attached caps size 0.2 mL
- Disposable lab coats
- Disposable powder-free lab gloves
- 0,2mL microtube racks
- Thermocycler

Mastermix

All primers pools should be diluted to a total of 10uM from the 100uM stock.

Components	***Amount (uL)***
Nuclease Free water	14,25
Q5 reaction buffer (5x)	5
dNTPs (10mM)	0,5
Primers for the pool 10uM	2,5
Q5 DNA polymerase	0,25

Prepare 2 mastermixes with the above components for the appropriate number of samples. In one tube add the primers of the pool A and in the other tube, add primers of the pool B.

After the mastermix is finished, pipette out 22.5uL of the mastermix into PCR microtubes, and then add in each tube 2.5ul of the appropriate cDNA.

Thermocycling parameters

Step	***Temperature***	***Time***	***Cycle number***
Initial denature	98ºC	30 seconds	1
Denature	98ºC	15 seconds	45
Annealing and extension	65ºC	5 minutes	
Hold	4ºC	∞	1

PROTOCOL FOR CHIKV SEQUENCING PCR

This is a protocol for CHIKV multiplex PCR reactions prior to the library preparation of the Oxford Nanopore sequencing.

Materials

- DENV-2 primers pool A and B
- Micropipettes and tips
- Microcentrifuge tubes size 1,5mL
- PCR microtubes with attached caps size 0.2 mL
- Disposable lab coats
- Disposable powder-free lab gloves
- 0,2mL microtube racks
- Thermocycler

Mastermix

All primers pools should be diluted to a total of 10uM from the 100uM stock.

Components	***Amount (uL)***
Nuclease Free water	15,75
Q5 reaction buffer (5x)	5
dNTPs [10mM]	0,5
Primers for the pool [10Um]	1,0
Q5 DNA polymerase	0,25

Prepare 2 mastermixes with the above components for the appropriate number of samples. In one tube add the primers of the pool A and in the other tube, add primers of the pool B.

After the mastermix is finished, pipette out 22.5uL of the mastermix into PCR microtubes, and then add in each tube 2.5ul of the appropriate cDNA.

Thermocycling parameters

Step	***Temperature***	***Time***	***Cycle number***
Initial denature	98ºC	30 seconds	1
Denature	98ºC	15 seconds	40
Annealing and extension	65ºC	5 minutes	
Hold	4ºC	∞	1

PROTOCOL FOR SEQUENCING PCR OF SARS-COV-2

This is a protocol for multiplex PCR reactions prior to the library preparation of the Oxford Nanopore sequencing.

Protocol version updated on *01 June 2021*

Materials

- primers pool A and B
- Micropipettes and tips
- Microcentrifuge tubes size 1,5mL
- PCR microtubes with attached caps size 0.2 mL
- Disposable lab coats
- Disposable powder-free lab gloves
- 0,2mL microtube racks
- Thermocycler

Mastermix

All primers pools should be diluted to a total of 10uM from the 100uM stock.

ARTIC NETWORK PROTOCOL – Quick , Josh, 2020

Components	***Volume (uL)***		***Components***	***Reduced Volume (uL)***
Nuclease Free water	12,75		Nuclease Free water	5,33
Q5 reaction buffer (5x)	5		Q5 reaction buffer (5x)	2,5
dNTPs (10mM)	0,5		dNTPs (10mM)	0,25
Primers for the pool 10uM	4		Primers for the pool 10uM	1,8
Q5 DNA polymerase	0,25		Q5 DNA polymerase	0,125

Prepare ***2*** mastermixes with the above components for the appropriate number of samples. In one tube add the ***primers*** of the pool ***A*** and in the other tube, add primers of the pool ***B***.
After the mastermix is finished, pipette out ***22.5uL (or 10 uL if using the reduced reaction)*** of the ***mastermix*** into PCR microtubes, and then add in each tube ***2.5ul*** of the appropriate cDNA.

Thermocycling parameters

Step	***Temperature***	***Time***	***Cycle number***
Initial denature	98ºC	30 seconds	1
Denature	98ºC	15 seconds	30
Annealing and extension	65ºC	5 minutes	
Hold	4ºC	∞	1