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artic-measles/400/v1.0.0 Protocol V.1

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ARTIC

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

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Disclaimer

For research use only.

Abstract

This protocol is how to generate the primer pools for artic-measles/400/v1.0.0. Then generate cDNA generation via LunaScript RT, and run the PCR reactions. Downstream library preparation relies on the ARTIC LoCost amplicon sequencing (SQK-NBD114).

Materials

Reagents:

	Component	Supplier	Part Number
	LunaScript® RT SuperMix Kit	NEB	M3010L
	Tris-EDTA (TE)		
	Nuclease-free water		
	Q5® Hot Start High-Fidelity 2X Master Mix	NEB	M0494S

Equipment:

- Microcentrifuge
- Vortex mixer
- Thermal cycler (Ideally one for prePCR and one for PCR)
- P1000, P200, P20 and P2 pipette
- Ice bucket
- Timer
- Qubit fluorometer or equivalent

cDNA Synthesis

30m

1

Note

A positive control can also be included which may be a synthetic RNA constructs or high-titre clinical sample which can be diluted. This can help monitor run performance.

2 Mix the following components in a PCR strip-tubes/plate. Gently mix by pipetting and pulse spin the tube to collect liquid.

	A	B
	Component	**Volume**
	LunaScript RT SuperMix (5X)	2 µL
	Template RNA	8 µL
	Total	**10 µL**

Components for RT reaction

Note

Viral RNA input from a clinical sample should be between Ct 18-35. If Ct is between 12-15, then dilute the sample 100-fold in water, if between 15-18 then dilute 10-fold in water. This will reduce the likelihood of PCR-inhibition.

Note

To prevent pre-PCR contamination the mastermix should be added to the PCR strip-tubes/plate in the **mastermix** cabinet which should should be cleaned with decontamination wipes and UV sterilised before and after use.

RNA samples should be added in the **extraction/sample addition** cabinet which should should be cleaned with decontamination wipes and UV sterilised before and after use.

3 Incubate the reaction as follows:

25 °C for 00:02:00
55 °C for 00:10:00
95 °C for 00:01:00
Hold at 4 °C

Primer pool preparation

1h

4 If making up primer pools from individual oligonucleotides (oligos)

Note

If using pre-pooled scheme. Please skip to Step 7

4.1 Briefly spin down lyophilised oligos to ensure pellet is at bottom of the tube.

4.2 Resuspend oligos in 1x Tris-EDTA (TE) to a of concentration [M] 100 micromolar (µM), vortex thoroughly and spin down.

4.3 Dilute the [M] 100 micromolar (µM) pools 1:10 in molecular grade water, to generate [M] 10 micromolar (µM) working primer stocks.

5

Sort all odd regions primers into one or more tube racks. Add 5 µL of each odd region primer to a

1.5 mL Eppendorf tube labelled "Pool 1 [M] 100 micromolar (µM) ". Repeat the process for all even region primers for Pool 2. These are your [M] 100 micromolar (µM) stocks of each primer pool.

Note

Primers should be diluted and pooled in the **mastermix** cabinet which should be cleaned with decontamination wipes and UV sterilised before and after use.

6

Note

For artic-measles/400/v1.0.0; there are 193 primers in Pool 1 and 181 primers in Pool2. Therefore, to ensure each primer is at ~ [M] 15 nanomolar (nM) use either; 7.3 µL of [M] 10 micromolar (µM) working primer stocks or 0.73 µL of [M] 100 micromolar (µM) primer stocks per 25 µL PCR reaction.

Multiplex PCR

4h

7 Add 2.5 µL cDNA to each of the PCR reactions, gently mix by pipetting and pulse spin the tube to collect liquid at the bottom of the tube.

Note

cDNA should be added in the **extraction and sample addition** cabinet which should be cleaned with decontamination wipes and UV sterilised before and after use.

7.1

	A	B	C
	Component	**Reaction 1**	**Reaction 2**
	Q5 Hot Start High-	12.5 µL	12.5 µL

	A	B	C
	Fidelity 2X Master Mix		
	Pool 1 (10µM)	7.3 µL	0 µL
	Pool 2 (10µM)	0 µL	7.3 µL
	Nuclease-free water	2.7 µL	2.7 µL
	Total	**22.5 µL**	**22.5 µL**

Note

To prevent pre-PCR contamination the **mastermix** for each pool should be made up in the **mastermix** cabinet which should should be cleaned with decontamination wipes and UV sterilised before and after use and aliquoted into PCR strip-tubes/plate

8 Set-up the following program on the thermal cycler: 30s

Step	Temperature	Time	Cycles
Heat Activation	98 °C	00:00:30	1
Denaturation	98 °C	00:00:15	25-35
Annealing	65 °C	00:05:00	25-35
Hold	4 °C	Indefinite	1

Quality Control

9 The methods of quality control are very dependant on what is available in the lab. You want to check PCR yield and product size.

Yield can be measured on NanoDrop, Qubits or other Fluorometers. Yields are typically between [M] 10 ng/uL to [M] 100 ng/uL .

For Qubit, see step 25 in linked protocol under Library Preparation.

For product size, electrophoresis gels or TapeStations can be used to measure. A single band should be present at the size of the amplicons (~400bp). Some smear at ~20-60bp is expected (due to primers) particularly in negatives and low input samples.

Library preparation

10

Protocol

NAME

ARTIC LoCost Amplicon Sequencing Protocol (SQK-NBD114)

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Preview