ARTIC NGS Primer Pool – User Protocol

Eurofins Genomics

Contents

1. Storage

Storage at room temperature for < 3 days is possible.

For long term store please store at -20°C. Do not freeze/thaw more than 5 times.

The primers are delivered liquid in TE buffer (ph 8). Concentration: 10 µM

2. Reverse Transcription

A Reverse Transcription have to be performed prior the ARTIC PCR.

For Reverse Transcription we recommend LunaScript RT SuperMix (5x) (Cat. No E3010L) cDNA synthesis kit (NEB):

Quick Protocol for generation of cDNA:

Lunascript Supermix	2.2 µL
Water	3.3 µL
RNA sample	5.5 µL
Total	11 µL

Thermal Profile:
2 min 25°C (primer annealing)
20 min 55°C (reverse transcription)
1 min 95°C (heat inactivation)
∞ 4°C

Use 2 x 2.5 µL of this cDNA directly in the RC-PCR reaction according to the protocol.

3. Recommended PCR Master Mix

For the PCR reaction we recommend the NEBNext Ultra II Q5 Master Mix (NEB, Cat. No M0544L)

4. Kit Components

ARTIC NGS Primer Pool – Mix 1	96 reactions
ARTIC NGS Primer Pool – Mix 2	96 reactions

5. Prepare PCR

Prepare the reagents as follows:

Thaw Oligo Mix 1 and Olig Mix 2 at room temperature.

After thawing, mix all tubes by flicking and then briefly centrifuge.

Prepare the mastermix for the two target enrichment reactions in a suitable vessel according to the following tables:

Mastermix PCR Pool 1:

Component	**Volume**
cDNA	4.25 µL
NEBNEXT ULTRA II Q5 MASTER MIX	6.25 µL
ARTIC NGS Primer Pool – Mix 1	2 µL
TOTAL	**12.5 µL**

Mastermix PCR Pool 2:

Component	**Volume**
cDNA	4.25 µL
NEBNEXT ULTRA II Q5 MASTER MIX	6.25 µL
ARTIC NGS Primer Pool – Mix 2	2 µL
TOTAL	**12.5 µL**

Mix by pipetting up and down 5 times and briefly centrifuge.

Note: If more than one reaction is set-up, consider an excess volume of 10% for master mix preparation.

Incubate the reaction vessels in a thermocycler according to following protocol:

Cycle step	Temp	Time	Cycles
Initial denaturation	98°C	30 sec	1
Denature	95°C	15 sec	35
Annealing/extension	63°C	5 min	
Hold	4°C	indefinitely	1

After amplification, combine the entire contents of “Pool 1” and “Pool 2” PCR reactions for each sample and control into a single tube, giving a volume of 25 µl.

Proceed with downstream library preparation and NGS applications.