***ARTIC Multiplex PCR Protocol***

**Scheme:** artic-inrb-mpox/400/v1.0.0

Due to the large number of primers included in each pool, the 100 µM primer pool stocks do not need to be diluted as this would result in too large a volume for the multiplex PCR reaction.

Therefore, the 100 µM primer pool stocks are added directly into the Multiplex PCR reaction mix.

***Multiplex PCR:***

1. Set up two PCR reactions per sample as follows in strip-tubes or plates:
   1. Depending on available reagents use Reaction Mix A for M0493 (Q5® Hot Start High-Fidelity DNA Polymerase) or Reaction Mix B for M0494 (Q5® Hot Start High-Fidelity 2X Master Mix).

**Reaction Mix A:**

|  |  |  |
| --- | --- | --- |
| ***Component*** | ***Reaction 1*** | ***Reaction 2*** |
| 5X Q5 Reaction Buffer | 5 µl | 5 µl |
| 10 mM dNTPs | 0.5 µl | 0.5 µl |
| Q5 Hot Start DNA Polymerase | 0.25 µl | 0.25 µl |
| Pool 1 (100 µM) | 2.2 µl | 0 µl |
| Pool 2 (100 µM) | 0 µl | 2.2 µl |
| Nuclease-free Water | 14.55 µl | 14.55 µl |
| ***Total*** | 22.5 µl | 22.5 µl |

***OR***

**Reaction Mix B:**

|  |  |  |
| --- | --- | --- |
| ***Component*** | ***Reaction 1*** | ***Reaction 2*** |
| Q5 Hot Start High-Fidelity 2X Master Mix | 12.5 µl | 12.5 µl |
| Pool 1 (10 µM) | 2.2 µl | 0 µl |
| Pool 2 (10 µM) | 0 µl | 2.2 µl |
| Nuclease-free Water | 7.8 µl | 7.8 µl |
| ***Total*** | 22.5 µl | 22.5 µl |

1. Gently mix by pipetting and pulse spin the tube to collect liquid at the bottom of the tube.
2. Add 2.5 µl cDNA (or DNA) to each of the PCR reactions, gently mix by pipetting and pulse spin the tube to collect liquid at the bottom of the tube.
3. Set up the following program on the thermal cycler:
   1. Cycle number should be 25 for Ct 18 -21 up to a maximum of 35 cycles for Ct 35.

|  |  |  |  |
| --- | --- | --- | --- |
| ***Step*** | ***Temperature*** | ***Time*** | ***Cycles*** |
| Heat Activation | 98 °C | 30 Seconds | 1 |
| Denaturation | 98 °C | 15 Seconds | 25 – 35 |
| Annealing | 65 °C | 5 minutes | 25 – 35 |
| Hold | 4 °C | ∞ | 1 |

The pools can now be combined and sequenced.

For ONT sequencing, we recommend following: One-pot native barcoding of amplicons v4 (LoCost) V.2



<https://dx.doi.org/10.17504/protocols.io.kxygxebydv8j/v2>