PCR protocol for Monkeypox virus (MPXV) sequencing

1. Solutions and reagents

- Primer MPXV (10 µM) – Pool 1 e Pool 2
- 5X Q5 Reaction Buffer (New England Biolabs)
- dNTPs (10 mM)
- Q5 Hot Start DNA Polymerase (New England Biolabs)
- Nuclease free water

2. Procedure description

a. Prepare the master mix to each pool in separate tubes according to the table below (Table A):

Table A: Master Mix PCR MPXV		
	Pool 1	**Pool 2**
Components	**Volume (µL)/ per sample**	**Volume (µL)/ per sample**
Nuclease free water	11.25	11.25
5X Q5 Reaction Buffer	5.0	5.0
dNTPs (10 mM)	0.5	0.5
Q5 Hot Start DNA Polymerase	0.25	0.25
Primer (10 µM)	3.0	3.0
Total	**20.0**	**20.0**

b. Distribute 20.0 µl of each pool in two separate plates (one plate to Pool 1 and another to Pool 2);
c. Add 5.0 µL of DNA in the corresponding wells of pool 1 and pool 2 plates;
d. Homogenize gently by pipetting (10x);
e. Seal the plate with adhesive;
f. Centrifuge at 1,500 x g for 1 minute;
g. Place the plates in thermal cyclers and incubated under the conditions described below.

HOLD 1	1 x	98°C for 1 minute
CYCLING	35 x	98°C for 20 seconds 65°C for 1 minute 72°C for 4 minutes
HOLD 2	1 x	72°C for 5 minutes
HOLD 3	∞	4°C

Proceed with pools 1 and 2 for genomic library preparation, according to the sequencing technology.