REVIEW OF WET LAB

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CENTRE FOR PATHOGEN GENOMICS

World Health Organization
Western Pacific Region

IPSN International Pathogen Surveillance Network
WHO hosted network

Mpox surveillance workflow.

A joint venture between The University of Melbourne and The Royal Melbourne Hospital

The core stages of this workflow are:

a) specimen collection
b) sample preparation
c) genome sequencing processing of sequencing results
d) sequence data interpretation and data sharing.

Wet Lab Training Goals

CENTRE FOR PATHOGEN GENOMICS | Doherty Institute | The University of Melbourne | The Royal Melbourne Hospital

A joint venture between The University of Melbourne and The Royal Melbourne Hospital

DAY 1

Sample preparation

- Pipetting skills refresher
- MPXV Panel (7 samples)

  Amplicon PCR Scheme: Artic-inrb-mpox/2500/V1.0.1
  2500 bp with Pool 1 and 2

DAY 2

Genome sequencing

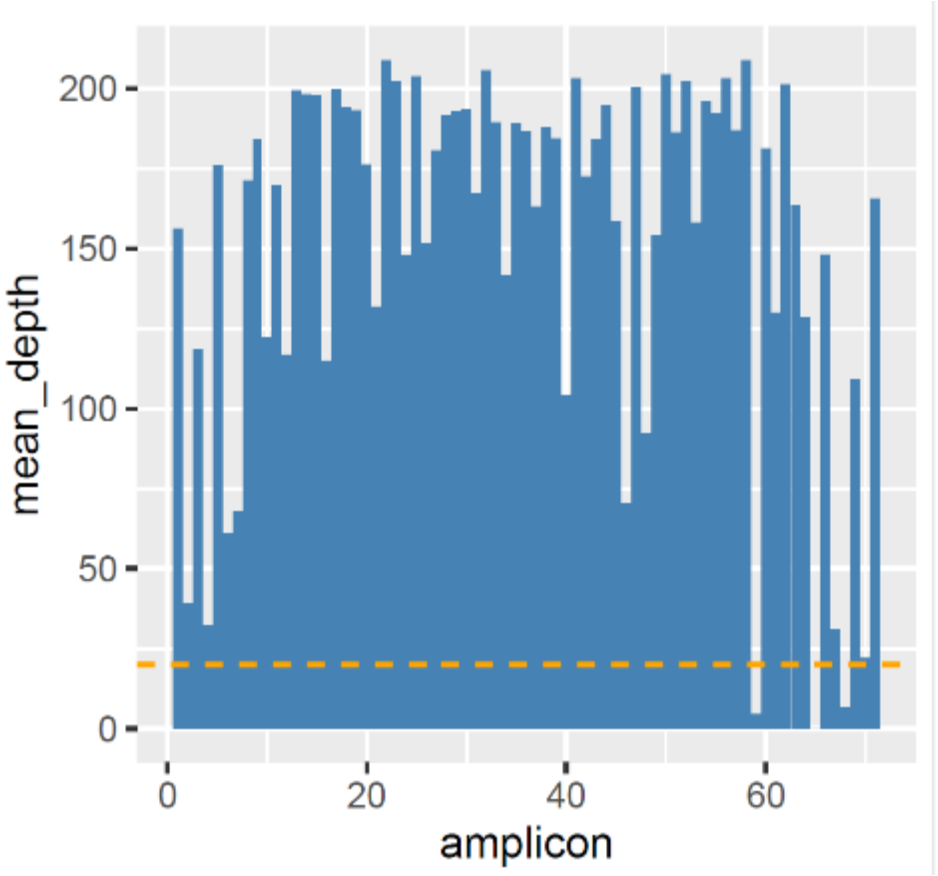
- Quantify amplicons
- Create a ONT library using Rapid Barcoding
- Quantify library
- Load and run libraries on a MinION R10.4.1 for <24 hours

Mpox Panel Results

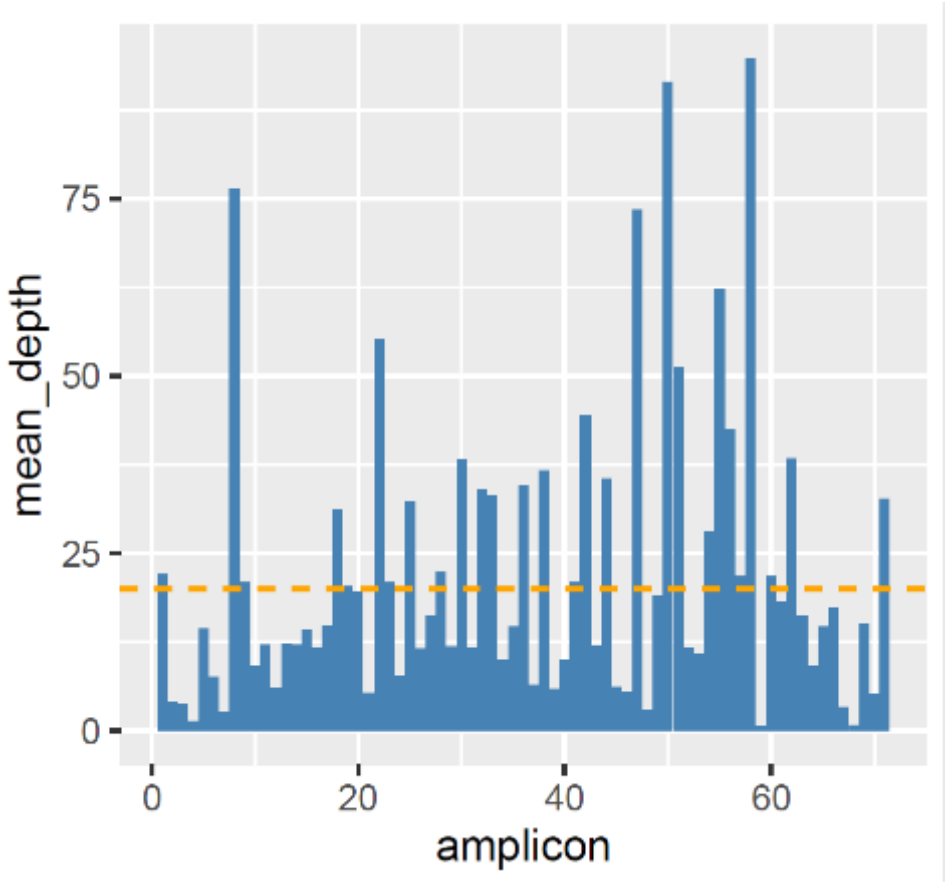
	Sample	Real Time	Swab Site	Clade	Lineage	Sequencing
1	MPOX	25.6	Lesion	IIb	F.2	Pass
2	MPOX	21.9	Lesion	IIb	F.2	Pass
3	HSV	Not Det	Isolate			Fail
4	MPOX	21	Ulcer	IIb	F.2	Pass
5	MPOX	29.1	Ulcer	IIb	F.2	Fail
6	MPOX	19	Unknown	IIb	F.2	Pass
7	NEGATIVE	Not Det				Fail

Mpox Sequencing

CT 25



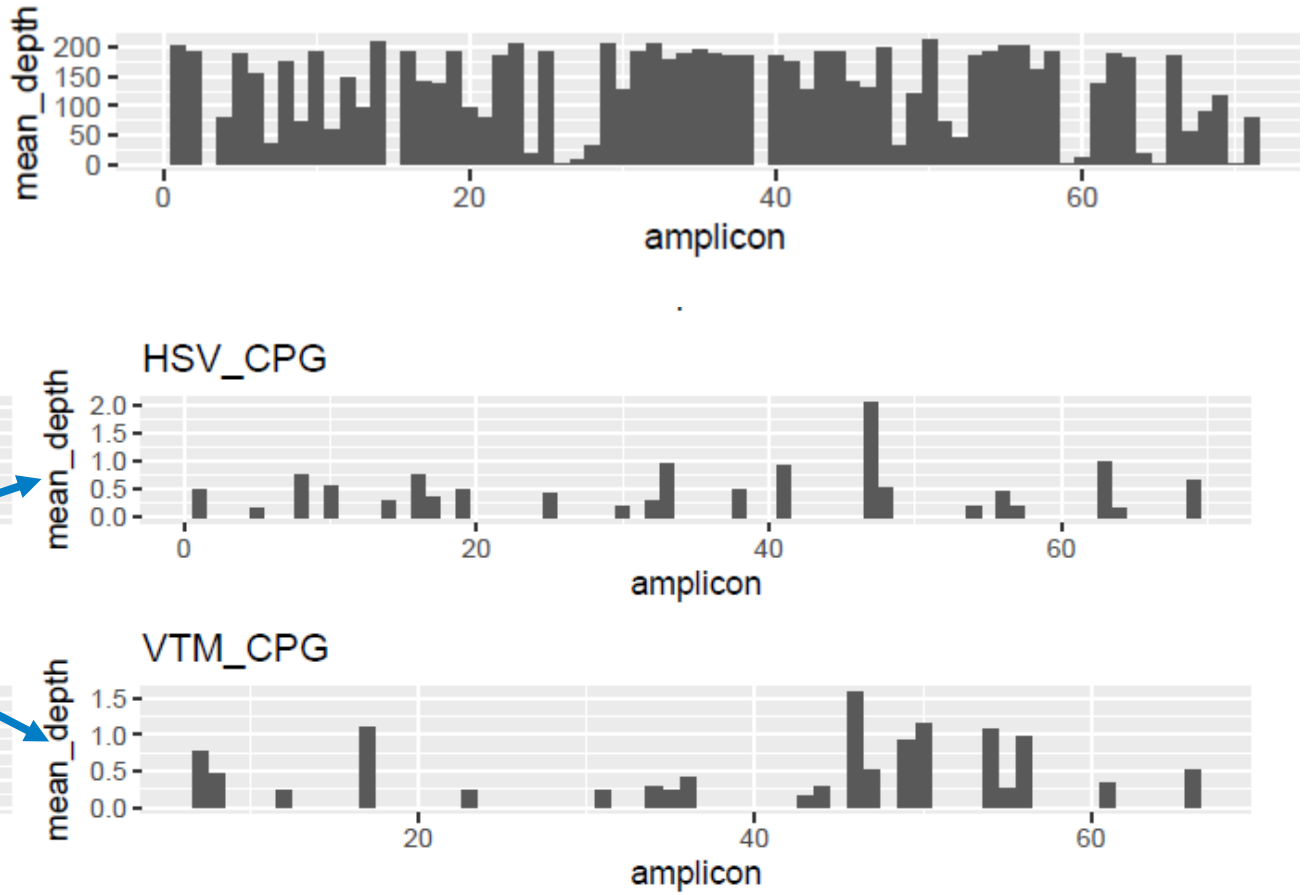
CT 30



Mpox Panel

Sample	Clade	Lineage	Ct	status
5	IIb	F.2	29.1	pass
3 - HSV	-	-	Not Det	FAIL
7 - VTM	-	-	Not Det	FAIL

Note: scale difference on HSV and VTM



Sequencing Approaches Mpox

EXAMPLE	SEQUENCING APPROACH
Clinical samples with low viral load (Ct > 28)	Amplicon-based WGS OR Target capture
Detection of structural variants, ITR variation	Amplicon-based WGS or Metagenomic sequencing with long-read technology
Accurate SNP calling and clade typing	Amplicon-based WGS
Validation of amplicon dropout or suspected deletions	Metagenomic sequencing or Target capture
High-throughput routine surveillance	Amplicon-based WGS
Field deployment with minimal infrastructure	Amplicon-based WGS with long-read technology
Wastewater or environmental samples	Metagenomic sequencing or Target capture with short amplicons Possibly: amplicon-based sequencing with short amplicons
Resource-limited labs with basic capacity	Amplicon-based WGS

Viral Genome Sequencing
Mpox

Genome sequencing from clinical samples with sufficient viral load can assist in determining MPXV **clades, subclades and lineages.**

In addition, sequencing may help identify or rule out transmission chains, provide contextualisation for resolving new international incursions or local circulation

PHLN
Public Health Laboratory Network

Public Health Laboratory Network laboratory case definition documents for nationally notifiable diseases within Australia.

MXPV and Humans Direct Testing

Multisite studies confirmed that polymerase chain reaction (PCR) testing of skin lesions has the highest yield (clinical sensitivity 91%–100%).

Direct sequencing of clinical samples containing MXPV possible

PCR amplicon

Hybridisation-probe capture

Metagenomics sequencing

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MPXV Publications - VIDRL

Received: 9 November 2022 | Accepted: 21 December 2022
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REVIEW

JOURNAL OF MEDICAL VIROLOGY WILEY

Mpox diagnostics: Review of current and emerging technologies

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Abstract

Mpox is a zoonotic disease caused by monkeypox virus (MPXV) from the *Orthopoxvirus* genus. Unprecedented transmission events have led to more than 70 000 cases reported worldwide by October 2022. The change in mpox epidemiology has raised concerns of its ability to establish endemicity beyond its traditional geographical locations. In this review, we discuss the current understanding of mpox virology and viral dynamics that are relevant to mpox diagnostics. A synopsis of the traditional and emerging laboratory technologies useful for MPXV detection and in guiding "elimination" strategies is outlined in this review. Importantly, development in MPXV genomics has rapidly advanced our understanding of the role of viral evolution and adaptation in the current outbreak.

KEYWORDS
diagnostics, genomics, monkeypox, mpox, orthopoxvirus, serology

ELSEVIER

The Lancet Microbe
Available online 7 October 2024, 100999
In Press, Corrected Proof ⓘ What's this?

Comment

Mpox genomics in outbreak control: challenges and limitations

Kathryn Edenborough [a b #], Ammar Aziz [a b #], Nicola Sexton-Oates [a b], Ivana Savic [a b], Eike Steinig [a b], Brendan Quinn [c], Mihaela Ivan [c], Alicia Arnott [a b], Leon Caly [a b ✉], Chuan Kok Lim [a b ✉]

JOURNAL OF MEDICAL VIROLOGY

RESEARCH ARTICLE | Open Access

Intra- and interhost genomic diversity of monkeypox virus

Mona L. Taouk ✉, Eike Steinig, George Taiaroa, Ivana Savic, Thomas Tran, Nasra Higgins, Stephanie Tran, Alvin Lee, Maxwell Braddick, Michael A. Moso, Eric P. F. Chow, Christopher K. Fairley ... See all authors

First published: 11 August 2023 | https://doi.org/10.1002/jmv.29029

Next Steps....

Mpox samples to be sequenced

Prioritize samples:

- **Unique samples** – some patients can be swabbed several times of the course of their infection
- Swabs from **high viral load sites** – lesions
- **Extract samples ASAP** and freeze in -80°C
- Batch runs for sequencing.

Mpox Panel Protocol

Next steps....

PCR 2500bp Tiling of Monkeypox Virus with ONT Rapid Barcoding

This protocol describes the procedure for PCR tiling of Monkeypox (MPXV) viral DNA samples. Multiplexing can occur for up to 40 samples onto a single run. It is an adaption of the protocol published by Oxford Nanopore Technologies using the Rapid Barcoding Kit 96 and Midnight RT PCR Expansion for barcoding and library preparation using ARTIC/INRB (Clinical) primers to generate amplicon (Quick Lab 2024).

Equipment and Consumables

Equipment

- Magnetic rack (1.5ml tubes)
- Microfuge/quick spin for tubes/pcr strips
- Vortex mixer (optional)
- Thermal cycler
- Qubit® Fluorometer
- Oxford MinION Mk1B/GridION

- Gloves (S,M,L)
- Safety Glasses
- Disposable gowns
- Tip waste sharps contai

Reagents

- Input DNA with known
- Q5® Hot Start HF 2x Ma
  purchased from New Er
- Monkeypox primers (INI
  Technologies. Pooled as
  stock) – CPG supplied
- Rapid Barcoding Kit 96:
  SQK-RBK110.96. The ki
  - Rapid Barcode Pla
  - AMPure XP Beads
  - Sequencing Buffe
  - Rapid Adapter F (
  - Elution Buffer (EB

Next Steps....
Mpox Sequencing

Run Controls

- Always have **a negative control** from extraction to sequencing.
- **Maximum of 48 samples** of Mpox on a ONT run (not including NTCs)

- **Positive control** = previous patient positive (CT<20)
  - Use this control over several runs to track PCR and sequencing quality

CPG supplied Primer Check – 1 x pos, 1 x negative, 1 x NTC. Check Qubit result.

1st Run Review with CPG

Completion of 1st ONT Mpox run.

Catch up with CPG team to review and reflect!

:)

Sample pretreatment

Library Preparation kits have input requirements

GOAL= greater proportion of pathogen reads in library

Pathogens can make up a small proportion of the original specimen

- **DNAse treatment**
- **Ampure bead clean up** (1:1)
- **Ribosomal RNA removal**
- **Nucleic Acid concentration** (Kits, elution volume, speed vac)
- **cDNA Synthesis** (First strand and Second Strand)

highly concentrated, purified ssDNA/RNA that is suitable

for subsequent library prep methods

Storage of NGS samples and reagents

Samples & Extracted material / cDNA

- -20°C (freeze/thaw considerations)
- “to be sequenced” box

Completed Library (plate) or

Pooled Library (tube)

- Freeze if not used immediately

Data

- Have backup hard Drives, S Drives etc.
- Detailed worksheets and sample details

Contamination in Library Prep

Opening more than one tube at a time

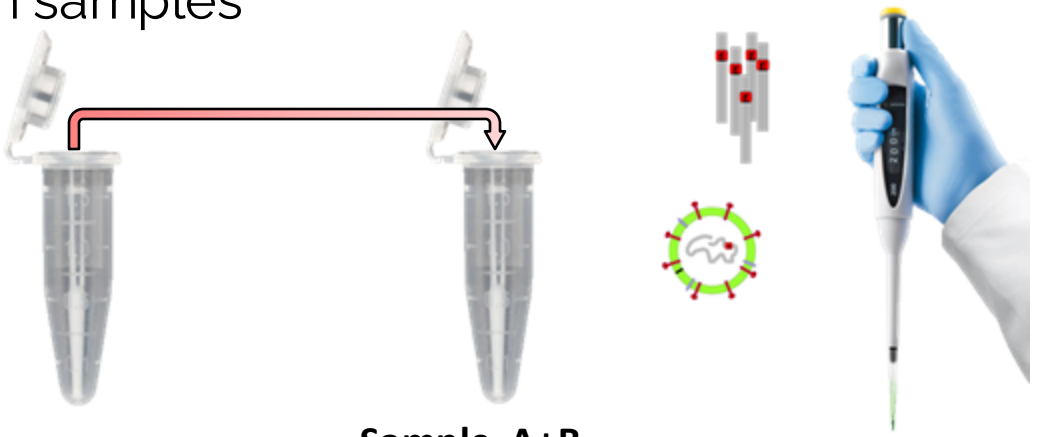
OR

Not changing gloves after a spill or pipetting error

OR

Not changing tips between samples

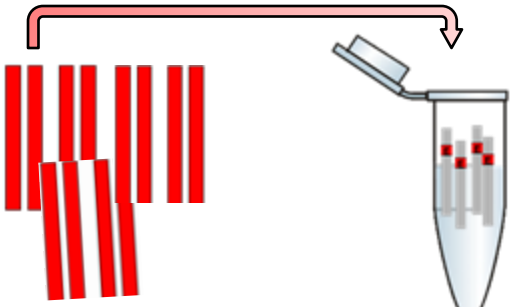
can lead to….



Sample A+B

AMPLIFIED MATERIAL CARRYOVER

Products from previous NGS
Not following unidirectional workflow



amplified PCR product

Unwanted mixture of libraries

Sample A

Genomics key goals

Performing NGS experiments accurately and reproducibly.

Requires a careful and considerate mode of working.

NGS is a highly sensitive technique.

=

Highly sensitive to contamination

=

Incorrect patient diagnosis or invalid run.

Sequencing with ONT

What can you sequence on ONT?

Strand sequencing method = nanopore sequences what ever is presented to it regardless of length

Any nucleic acid – Genomic = bacterial, fungal, metagenome

PCR amplicons – Tiled SARS CoV- 2, RSV, universal amplicons for segmented viral genomes e.g. flu.

PCR panels - AMR detection

RNA – Viral genomes, transcriptomes, cDNA

Targeted sequencing = cas9 enrichment, hybridization enrichment, adaptive sampling.

Illumina Microbial Amplicon Prep (IMAP)

A joint venture between The University of Melbourne and The Royal Melbourne Hospital

"Versatile library preparation solution for public health surveillance and microbiology Research"

Supports user-designed primers to sequence pathogens of public health concern

- Enables high-quality genome-wide coverage across multiple microbial species
- Accommodates DNA and RNA inputs from a range of sample sources and type

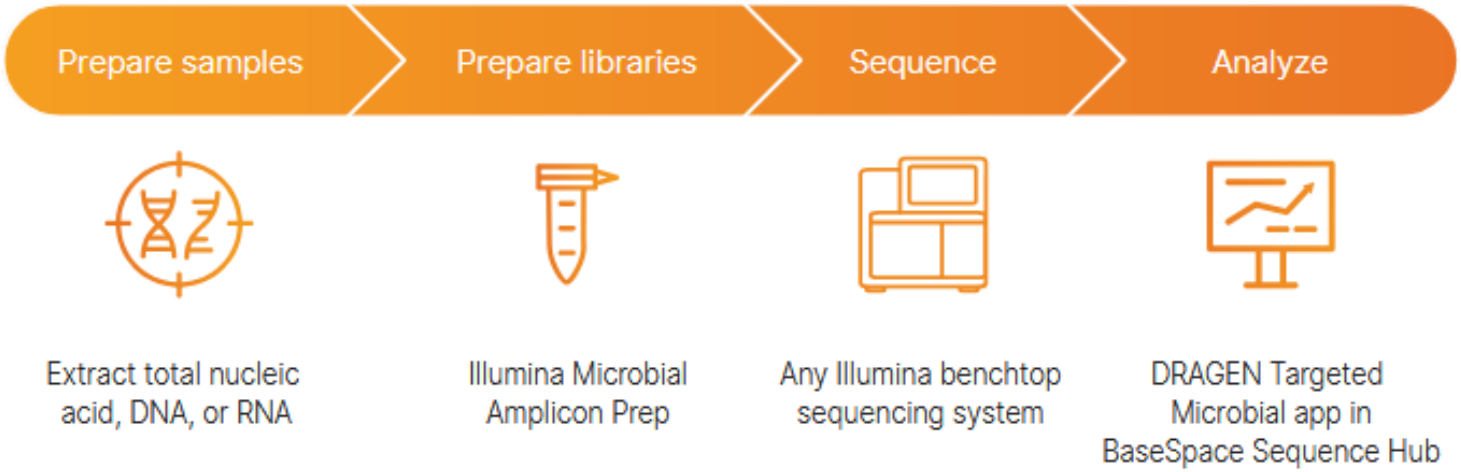


Image source: www.illumina.com

Illumina Microbial Amplicon Prep (IMAP)

This kit is based on Illumina COVIDSeq Assay workflow:

cDNA conversion, amplification and library preparation

Amplicon lengths of 400 base pairs are recommended.
but longer amplicons may be necessary with some targets.

Illumina Tested IMAP protocols:

- Chikungunya
- Dengue
- **Mpox**
- RSV
- Zika

Product	Catalog no.
Illumina Microbial Amplicon Prep (48 samples)	20097857

IMAP and Mpox Scheme

The kit is compatible with lab-designed primers or commercially available **primer sets which are purchased separately.**

Scheme	Amplicon size [bp]	# Primer pairs	Optimal Ct	Reference for primer design	Sequencing Platform (validated)	Clade Ib coverage
Chen et al. 2023	Average 200	163	<31	IIb (MT903345)	Illumina	85-90%

Also known as the “Yale” or “Vogels” scheme

Development of an amplicon-based sequencing approach in response to the global emergence of mpox

Nicholas F. G. Chen, Chrispin Chaguza, Luc Gagne, Matthew Doucette, Sandra Smole, Erika Buzby, Joshua Hall, Stephanie Ash, Rachel Harrington, Seana Cofsky, Selina Clancy, Curtis J. Kapsak, Joel Sevinsky, [...], Chantal B. F. Vogels

[view all]

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THANK YOU

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