# Schedule DAY 2

| TUESDAY | ACTIVITY | PRESENTER |
|---|---|---|
| 9.00 – 9.10 | Review of Day 1 | Jean |
| 9.10 – 10.00 | Lab: Amplicon QC | Louise |
| 10.00 – 10.30 | Morning tea | |
| 10.30 – 12.30 | **Lab:** Library Preparation & Loading | Jean |
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| 13.30 – 14.15 | **Lab:** Flow cell loading practice and Run Reviewing | Louise |
| **14.15 – 14.30** | **Lecture: REVIEW OF LABORATORY WORKFLOW** | **Jean** |
| 14.30 – 15.30 | Panel: Ask us anything! | |
| 15.30 – 16.00 | Afternoon Tea | |
| 16.00 – 17:00 | **Lecture:** MPXV Illumina Sequencing Options | Jean |

# REVIEW OF LABORATORY WORKFLOW

Jean Moselen
Senior Medical Scientist
Victorian Infectious Disease Laboratory (VIDRL)

CENTRE FOR PATHOGEN GENOMICS

World Health Organization
Western Pacific Region

IPSN International Pathogen Surveillance Network
WHO hosted network

# 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.

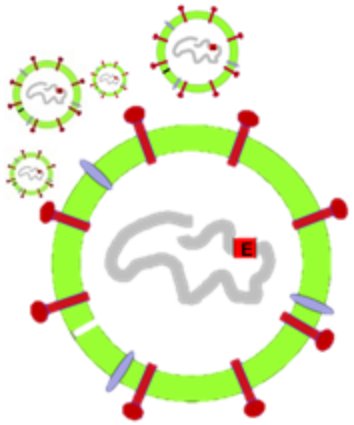
# Mpox surveillance workflow.



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.

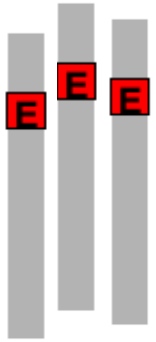
# Location of work in the NGS lab is important



Virus in clinical sample

INFECTIOUS

Extraction



Isolated genetic material (template)

NON - INFECTIOUS

Freeze after extraction

Amplification

Perform PCR

Post amplification

Library prep methods

Discard amplicon

## WHERE is this work done?

# Next Generation Sequencing

## Zones of work


**Work can be done over days/weeks**

**Safe stopping points**

**Emphasis is always on the avoidance of contamination with each step of the workflow…**

**WET LAB**

**DRY LAB**

Any kind of NGS technology generates a significant amount of data.

# UNIDIRECTIONAL WORKFLOW

## ONE DIRECTION 1 → 2 → 3 → 4 → 5 → 6

PRE-PCR

"CLEAN"

### 1 Extraction Zone
Processing of sample
Isolation of genetic material (template)

### 2 Mastermix Room
PCR Master mix
Reagent preparation and aliquoting

### 3 Template addition
Addition of template to Library Prep reagents

POST-PCR

"DIRTY"

### 4 Amplification Room
PCR thermal cycling
Amplification of genetic material/libraries

### 5 Library Preparation
PCR thermal cycling
Amplicon manipulation
Quantification of Libraries

### 6 Sequencing
Dilution of pooled libraries
Cartridge/Flow cell Loading

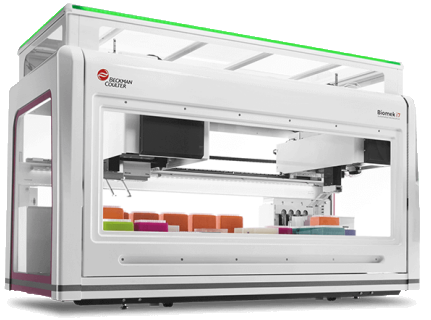
CENTRE FOR PATHOGEN GENOMICS
Doherty Institute
THE UNIVERSITY OF MELBOURNE
The Royal Melbourne Hospital
A joint venture between The University of Melbourne and The Royal Melbourne Hospital

# NGS lab Infrastructure

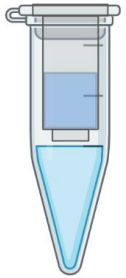
## Wet lab – Equipment vs throughput

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

**HIGH <96 samples +** — 96 well plate format

| EXTRACTION | QUANTIFICATION | ANALYSER | LIQUID HANDLER |
|---|---|---|---|
|  |  |  |  |
| King Fisher Apex<br>+Tan Bead Maelstrom™ 9610 | CLARIOstar Plus | TapeStation 4200 | Biomek i5 x 3 & i7 x 2 |

**LOW <48 samples**

| | | | |
|---|---|---|---|
|  |  |  |  |
| Column extractions | QuBit 4 | TapeStation 2200 | EpMotion<br>Manual library prep |

# NGS lab Infrastructure

## Wet lab – Sequencer vs throughput

| Data Output low(er) | | Data Output high(er) | |
|---|---|---|---|
| GridION | iSeq100 | NextSeq 1000/2000 | |
|  |  |  | Deep Sequencing = lots of data |
| Amplicon schemes | + Bait capture low sample # | | |
| MiSeq | | PromethION 2 Solo | Metagenomics<br>Bait capture<br>Complex samples<br>Adaptive sampling |
|  | Amplicon schemes<br>Bait capture<br>Complex samples |  | |

# Routine cleaning
## Equipment and surfaces

All areas during lab procedures (particularly lab bench, PCR workstation and BSCII)

**Scheduled routine cleaning. Daily/weekly basis.**

Use a DNAAWAY followed by 80% ethanol to wipe down your:

**Bench top** – Spray/wipe
**Pipettes** – wipe only
**Centrifuge and Vortex** – wipe only
**Racks** – Spray/soak (DET-SOL 5000)
**Freezer Doors, cabinets, drawers** – spray/wipe
**Thermocycler lid and buttons** - wipe

# Genomics contamination

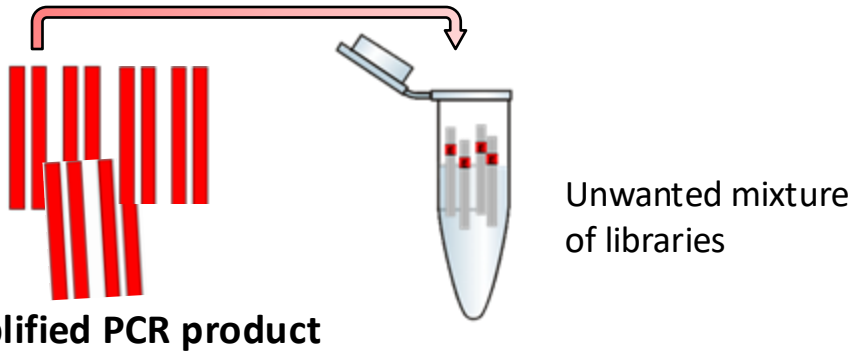
**The best way to deal with the risk of contamination is to take every precaution to prevent it from happening!**

Environmental  checks

contamination by exogenous DNA or RNA must be controlled to minimize the risk of faulty results.

## AMPLIFIED MATERIAL CARRYOVER

Products from previous NGS
Not following unidirectional workflow

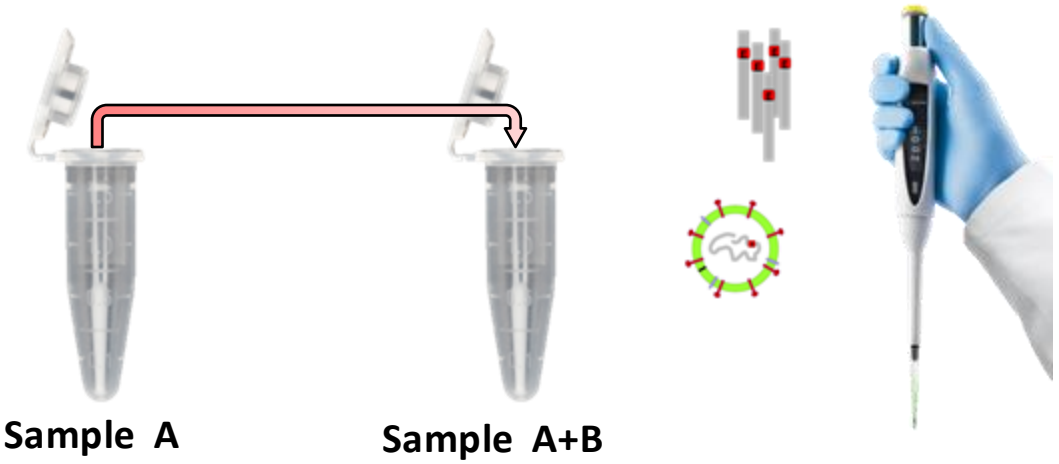


# Contamination in Library Prep

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 prep area, extraction, during library prep (pre barcoding)**



# 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.



# 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.

# Sample Input

**Library Preparation kits have input requirements**

- Sample volume, concentration of genetic material, sample type


**Accurate quantification of nucleic acid is critical for NGS methods**

**Fluorometric (Qubit)–** Targets specific type nucleic acid using a fluorophore and detected by a fluorometer, greater sensitivity. Less impacted by contaminants Qubit.
Binds = specific detection of molecules  e.g. RNA or DNA

# Sample pretreatment

## Library Preparation kits have input requirements

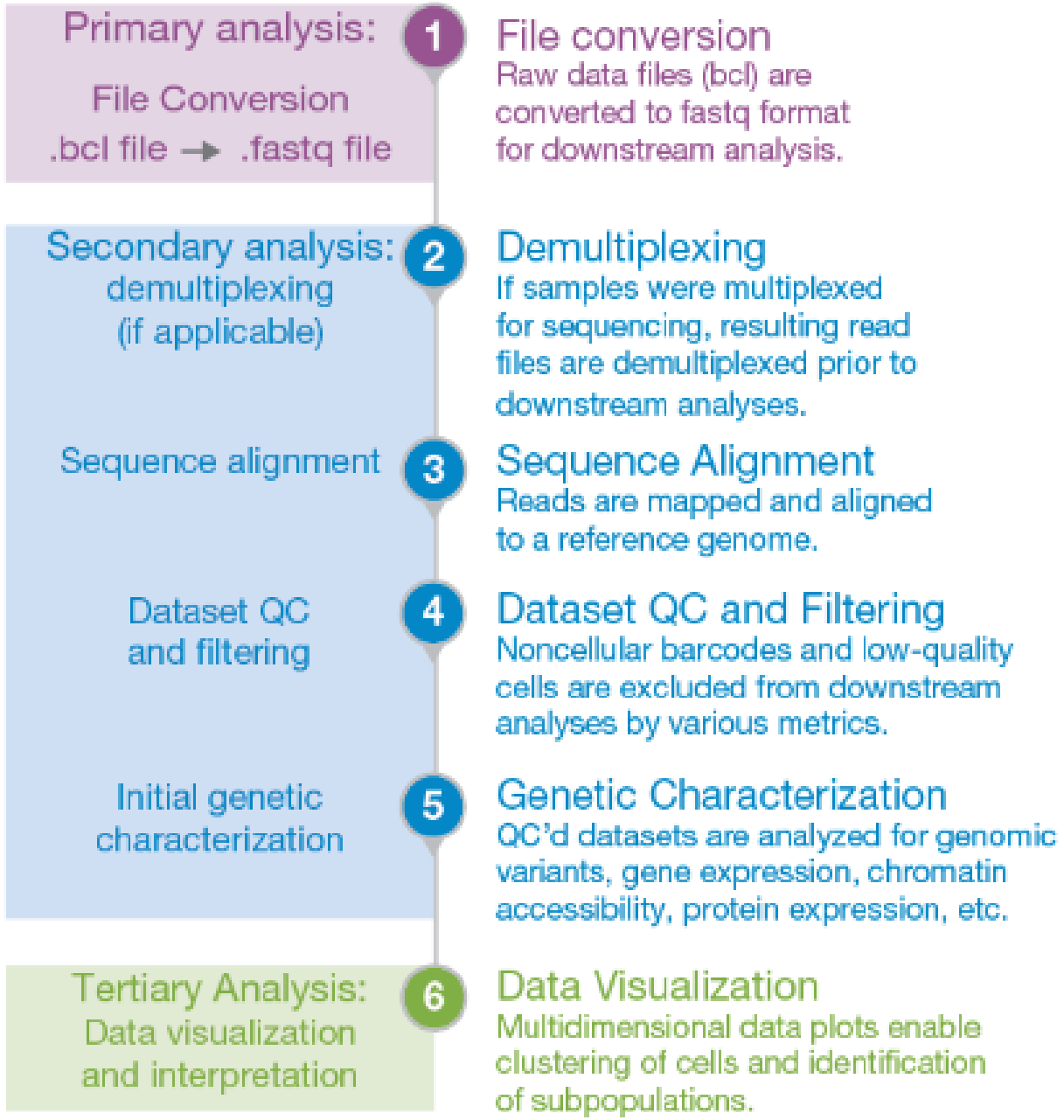
**GOAL=** greater proportion of pathogen reads in library

**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

# Bioinformatics



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