Doherty Institute

THE UNIVERSITY OF MELBOURNE

The Royal Melbourne Hospital

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

5 – Hands on – QC Consensus

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Recap

We ran the MPXV ONT pipeline which generated consensus sequences for our samples. Use the pipeline outputs to perform:

- Quality control of samples must be performed before downstream analysis.
- Exercise: assess sequence quality for different use cases

Viral Assembly Metrics

- Coverage plot
- Per amplicon depth plots
- Percent Genome Called
- SNP count and distribution
- Informative bases
- Mapped reads
- GC content

MPXV ONT Workflow – built in QC

Built in QC to simply your life

Plots:

- Genome depth plots
- Amplicon depth plots

Metrics:

- Percent genome covered by at least 20x
- Table of amplicon depths

Practice – QC Analysis of your data

Perform QC on the data you generated and answer these questions:

1. Look at the metrics for negative control sample(s) and compare to the positive control. How well did you perform in the lab? (Hint: how many reads aligned, what is the genome coverage?)
2. Find and collate the mean depth and genome coverage. Which sample would you pass/fail? And why (explain the thresholds you used)
3. For 'Pass' samples - which would you use for MPXV detection, phylogenetic analysis, lineage calling?
4. Did any sample produce a strange consensus? Tell everyone we want to see!