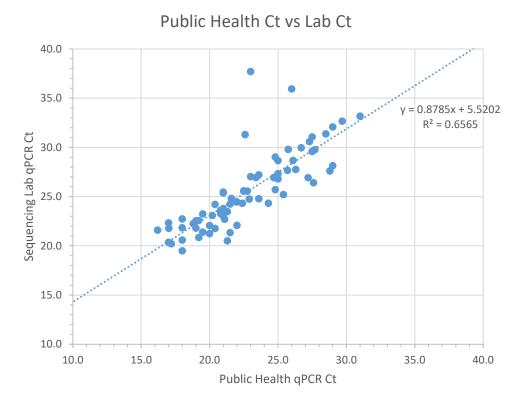
## **Supplementary Data**

## Global Mpox Lineage Discovery and Rapid Outbreak Tracking with Nanopore Sequencing

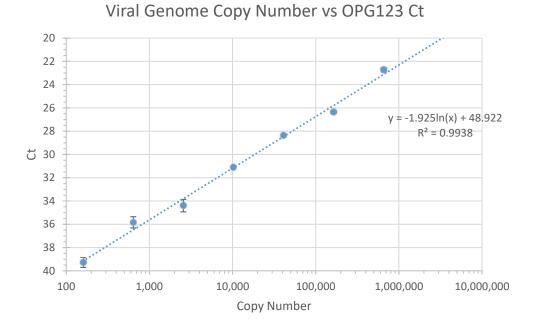
Michael S. Bosmeny<sup>1</sup>, Adam A. White<sup>1,†</sup>, Adrian A. Pater<sup>2,†</sup>, Jennifer Crew<sup>3</sup>, Joshua Geltz<sup>3</sup>, and Keith T. Gagnon<sup>1,2,\*</sup>

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 <sup>2</sup> Department of Chemistry and Biochemistry, Southern Illinois University, Carbondale, IL, USA.
 <sup>3</sup> Illinois Department of Public Health, Springfield, IL, USA.
 <sup>†</sup> These authors contributed equally.
 \* Corresponding Author: ktgagnon@siu.edu

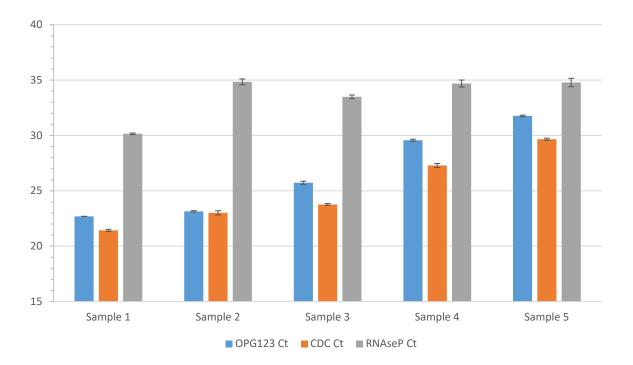
## **Supplementary Figures**



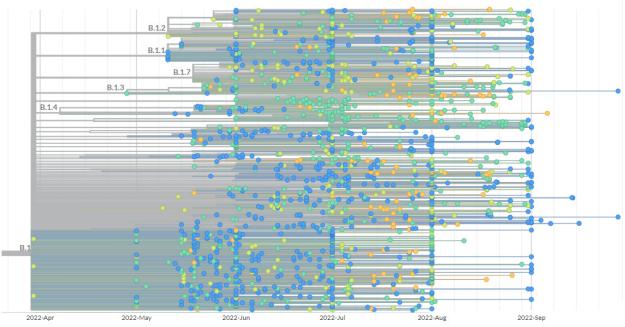
**Supplementary Figure S1**: Comparison of qPCR Ct values from Illinois Department of Public Health and those generated using OPG123 qPCR probes.



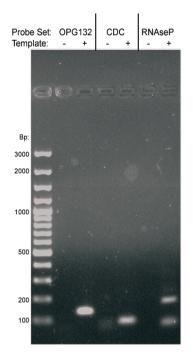
**Supplementary Figure S2**: Comparison of number of copies of a DNA gBlock and the qPCR Ct values generated from them using the OPG123 qPCR probe set.



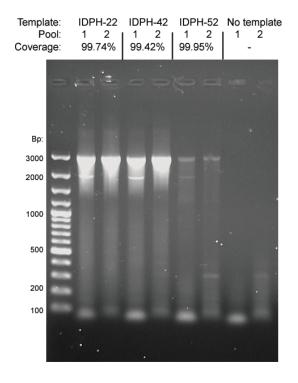
**Supplementary Figure S3**: Ct values for five different mpox samples, using three different qPCR probes. Each sample/probe combination was replicated three times.



**Supplementary Figure S4**: Phylogenetic tree showing Clade IIb, Lineage B.1 mpox sequences. Color of each sequence indicates status of indicated deletion starting at base 179077. Teal: no deletion. Lime-green: Deletion to base 179094 (17 bases removed). Blue: Deletion to base 179148 (71 bases removed). Orange: Deletion to base 179175 (98 bases removed). Each lineage follows no clear pattern in deletions, indicating this could be an artifact of sequencing method, rather than actual deletion.



**Supplementary Figure S5**: 1% agarose gel showing the positive and negative control results for three qPCR probe sets. Ladder size markers are shown on the left.



**Supplementary Figure S6**: 1% agarose gel showing three randomly-selected multiplex PCR samples, along with a negative control (no template). Final sequence coverage of each sample is also shown. Ladder size markers are shown on the left.

## **Table S1**: NCBI Accession Numbers for Mpox Genomes.

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MPXV-USA-IL-IDPH-025         ()           MPXV-USA-IL-IDPH-026         ()           MPXV-USA-IL-IDPH-026         ()           MPXV-USA-IL-IDPH-028         ()           MPXV-USA-IL-IDPH-029         ()           MPXV-USA-IL-IDPH-030         ()           MPXV-USA-IL-IDPH-031         ()           MPXV-USA-IL-IDPH-033         ()           MPXV-USA-IL-IDPH-033         ()           MPXV-USA-IL-IDPH-033         ()           MPXV-USA-IL-IDPH-033         ()           MPXV-USA-IL-IDPH-034         ()           MPXV-USA-IL-IDPH-035         ()           MPXV-USA-IL-IDPH-036         ()           MPXV-USA-IL-IDPH-037         ()           MPXV-USA-IL-IDPH-038         ()           MPXV-USA-IL-IDPH-040         ()           MPXV-USA-IL-IDPH-041         ()           MPXV-USA-IL-IDPH-041         ()           MPXV-USA-IL-IDPH-041         ()           MPXV-USA-IL-IDPH-041         ()           MPXV-USA-IL-IDPH-041         ()           MPXV-USA-IL-IDPH-042         ()           MPXV-USA-IL-IDPH-044         ()	OP536686	
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MPXV-USA-IL-IDPH-028         ()           MPXV-USA-IL-IDPH-029         ()           MPXV-USA-IL-IDPH-030         ()           MPXV-USA-IL-IDPH-031         ()           MPXV-USA-IL-IDPH-032         ()           MPXV-USA-IL-IDPH-033         ()           MPXV-USA-IL-IDPH-033         ()           MPXV-USA-IL-IDPH-035         ()           MPXV-USA-IL-IDPH-036         ()           MPXV-USA-IL-IDPH-037         ()           MPXV-USA-IL-IDPH-038         ()           MPXV-USA-IL-IDPH-039         ()           MPXV-USA-IL-IDPH-040         ()           MPXV-USA-IL-IDPH-041         ()           MPXV-USA-IL-IDPH-041         ()           MPXV-USA-IL-IDPH-041         ()           MPXV-USA-IL-IDPH-041         ()           MPXV-USA-IL-IDPH-043         ()           MPXV-USA-IL-IDPH-044         ()	OP536687 OP536688	https://www.ncbi.nlm.nin.gov/nuccore/OP536688 https://www.ncbi.nlm.nih.gov/nuccore/OP536688
MPXV-USA-IL-IDPH-029         ()           MPXV-USA-IL-IDPH-030         ()           MPXV-USA-IL-IDPH-031         ()           MPXV-USA-IL-IDPH-032         ()           MPXV-USA-IL-IDPH-033         ()           MPXV-USA-IL-IDPH-033         ()           MPXV-USA-IL-IDPH-034         ()           MPXV-USA-IL-IDPH-035         ()           MPXV-USA-IL-IDPH-036         ()           MPXV-USA-IL-IDPH-037         ()           MPXV-USA-IL-IDPH-038         ()           MPXV-USA-IL-IDPH-039         ()           MPXV-USA-IL-IDPH-0401         ()           MPXV-USA-IL-IDPH-041         ()           MPXV-USA-IL-IDPH-041         ()           MPXV-USA-IL-IDPH-041         ()           MPXV-USA-IL-IDPH-041         ()           MPXV-USA-IL-IDPH-042         ()           MPXV-USA-IL-IDPH-044         ()	OP536688 OP536689	
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