Supplementary materials

Evaluation of Optimal Pooling Strategies for Mpox virus Genomic Surveillance Using the Illumina Viral Surveillance Panel on the iSeq100 Platform in Outbreak Settings

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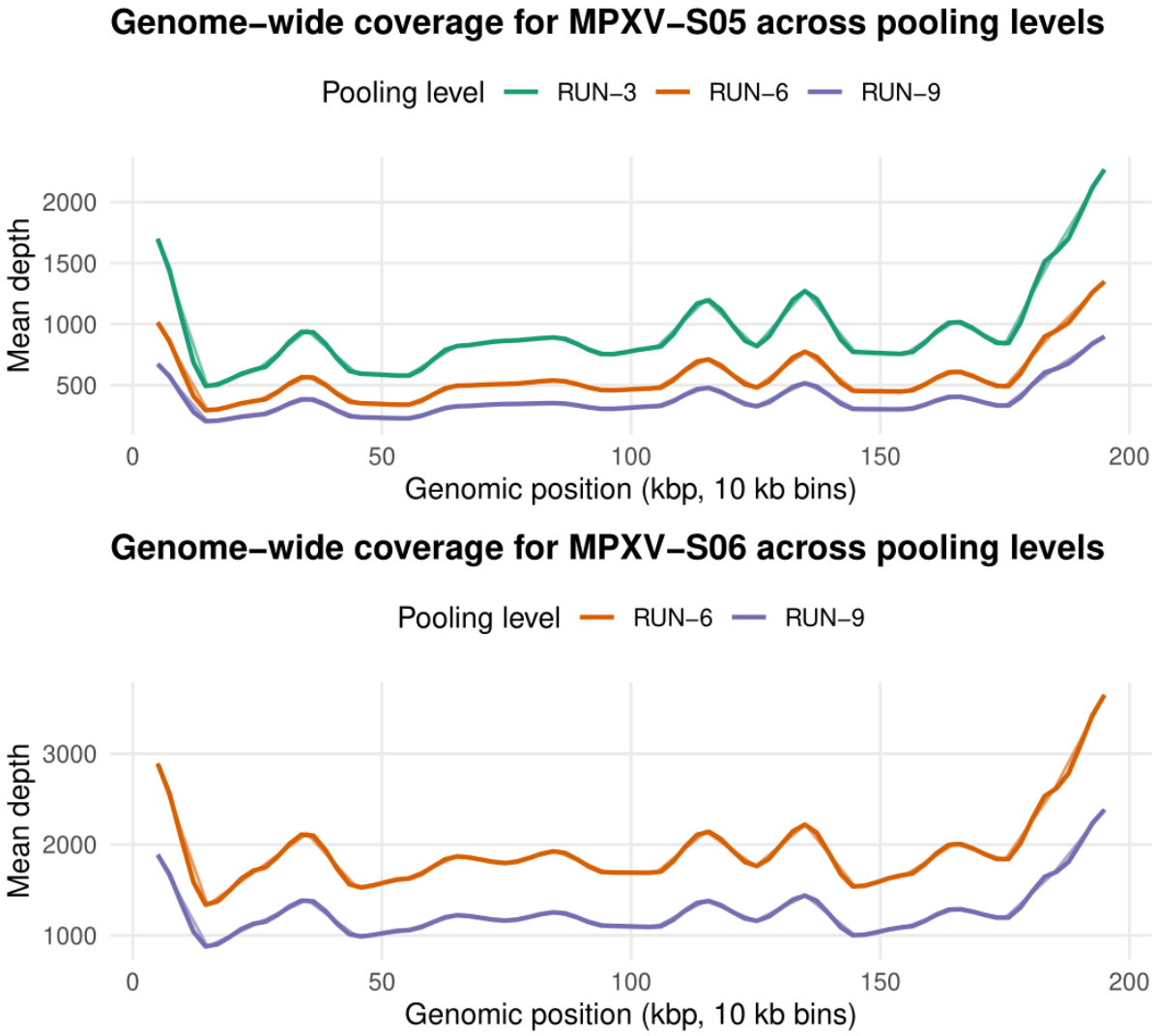


Supplementary Figure S1: Genome-wide coverage across pooling levels for MPXV-S05 and MPXV-S06 specimens. *Mean sequencing depth is plotted across 10 kb genomic bins for two representative samples. For MPXV-S05 (top panel), coverage decreases with increasing pooling level: RUN–3 (green) yields the highest depth, followed by RUN–6 (orange) and RUN–9 (purple). For MPXV-S06 (bottom panel), only RUN–6 and RUN–9 are shown, with RUN–6 consistently outperforming RUN–9. In both samples, coverage peaks are observed at the genomic termini, reflecting conserved enrichment patterns across pooling conditions.*

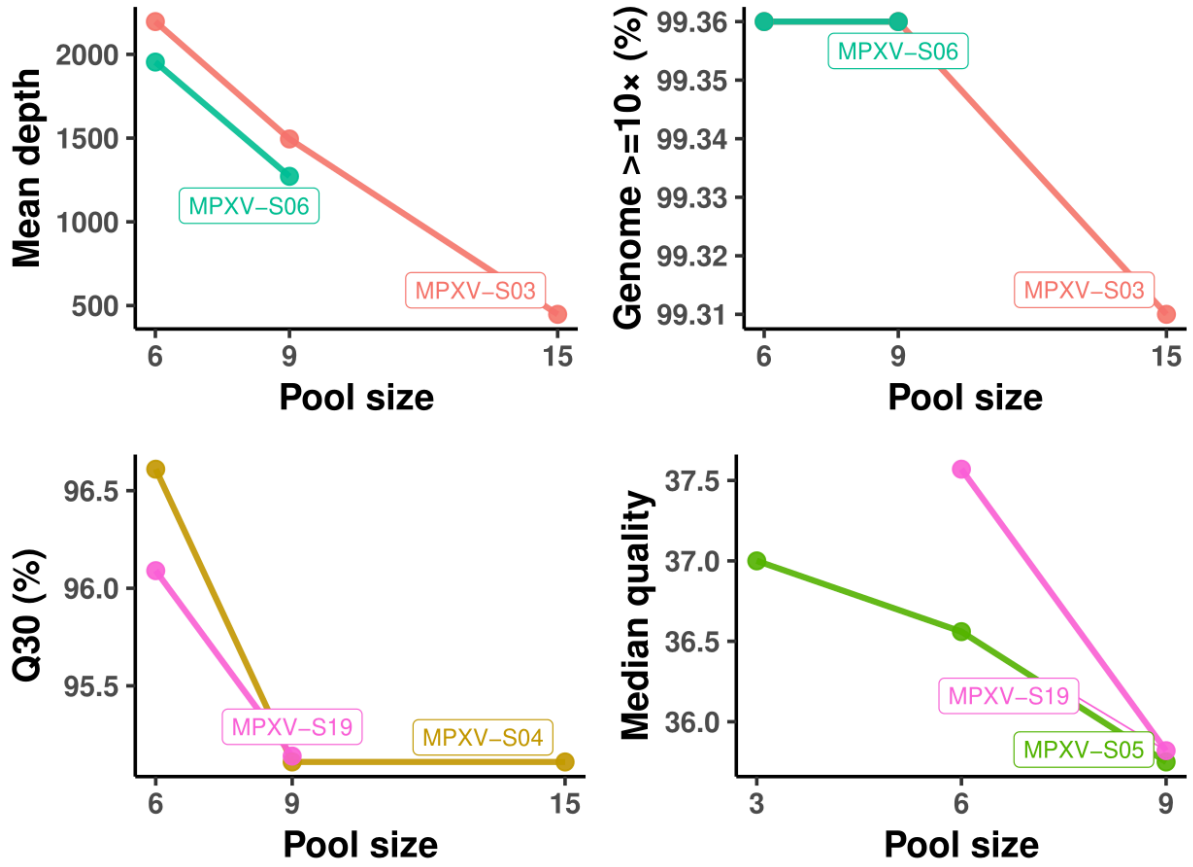
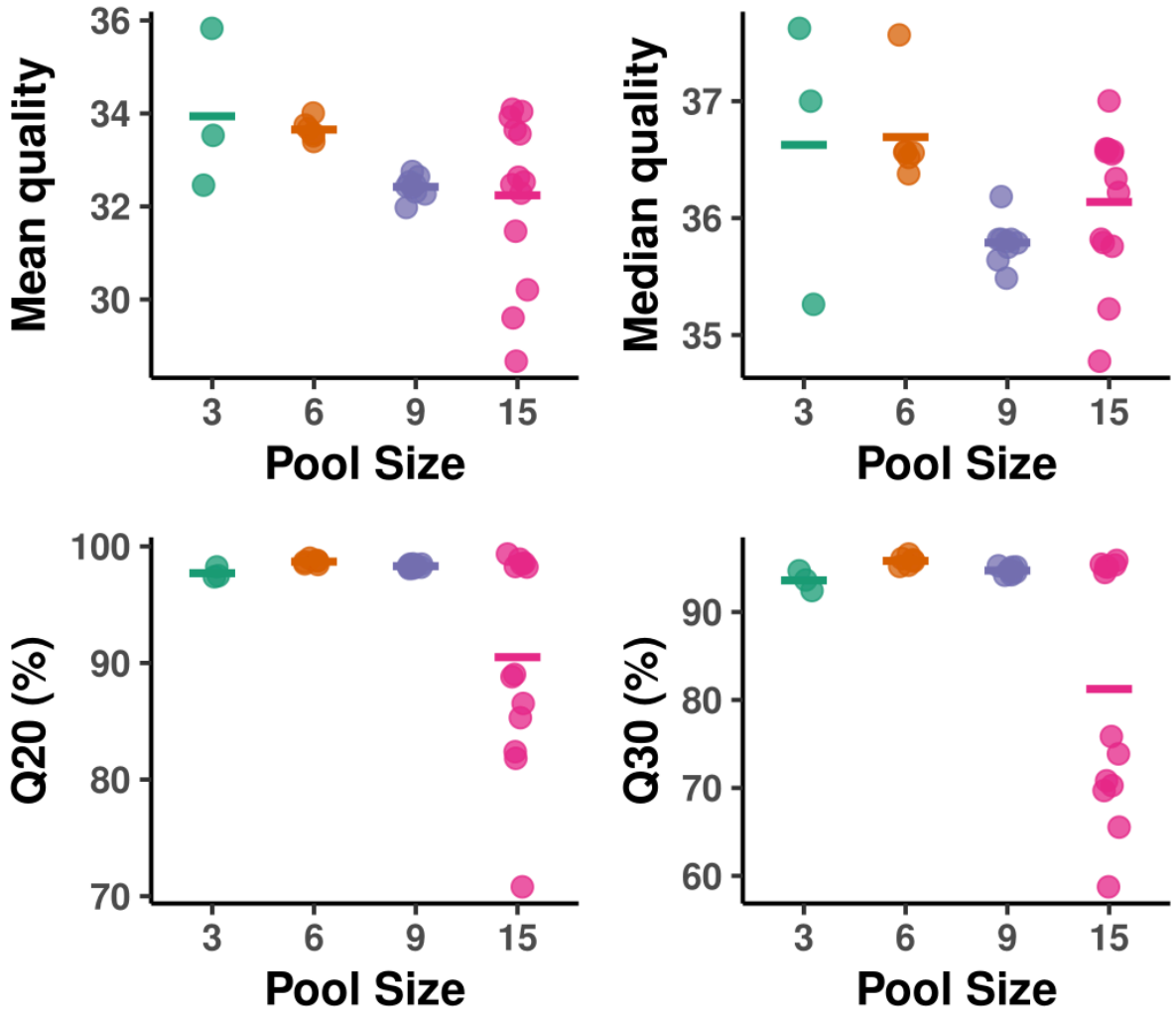


Figure S2. Effect of pooling level on sequencing quality metrics for repeated MPXV specimens. Representative samples were repeatedly included across different pooling levels to assess protocol robustness. Mean depth (top-left), genome coverage ≥10× (top-right), Q30 percentage (bottom-left) and median base quality (bottom-right) show only marginal variation (<1% in most cases) as pool size increases. These results suggest that sequencing quality remains largely stable across pooling levels.



Supplementary Figure S3: Read level quality metrics across pooling levels. *Distribution of mean quality score, median quality score, and the proportion of bases with Q20 and Q30 across RUN-3, RUN-6, RUN-9 and RUN-15.*