Supplementary Materials

Development and Validation of LAMP Assays for Distinguishing MPXV Clades with Fluorescent and Colorimetric Readouts

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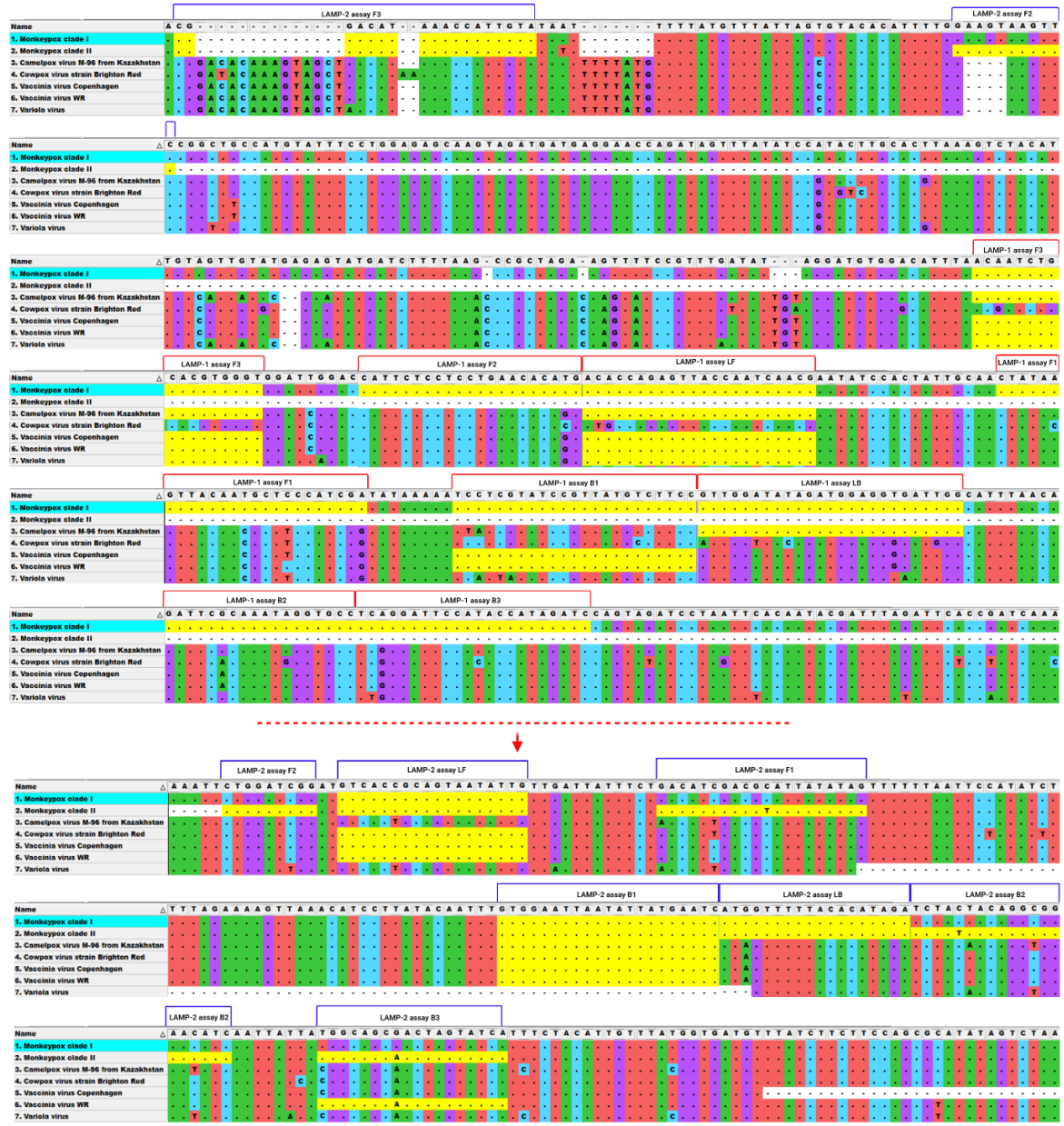
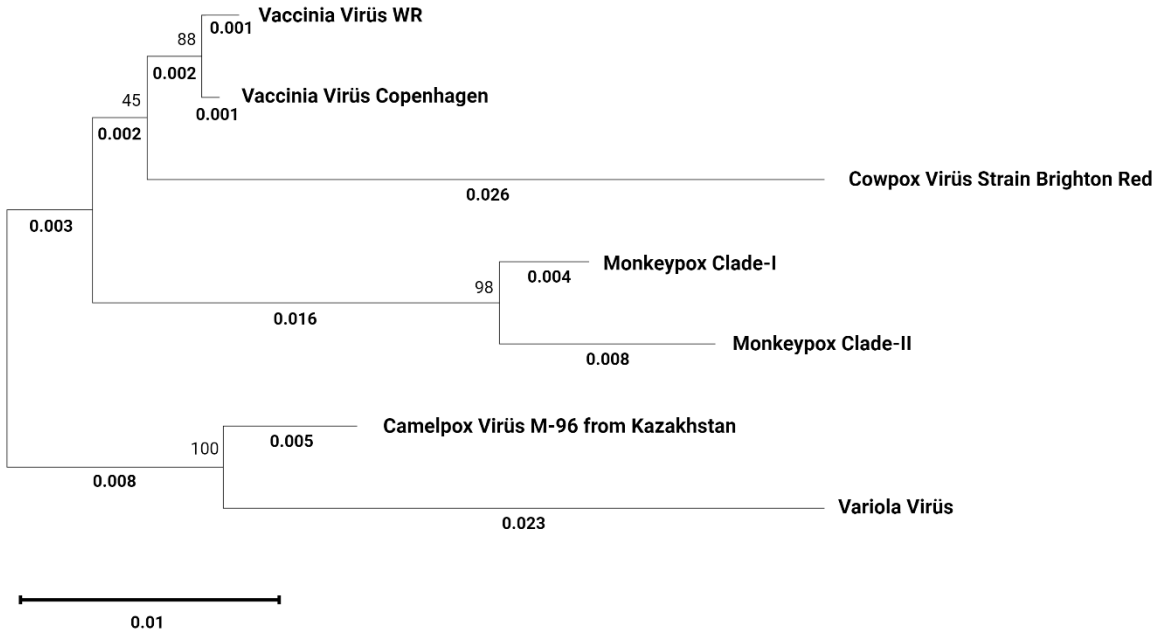


Supp Figure S1 Genetic map matching with MPXV-Clades and *Poxviridaes* family members and primer locations of LAMP assays on the map. The deletion found in Clade-II is not found in other Poxviridae family members. The LAMP-2 assay (R- Duplex DARQ-2 probe) can distinguish Clade-II from both Clade-I and *Poxviridae* family members (white-marked regions indicate deletions). All primers for the LAMP-1 assay were selected from the region present in the Clade-I genome sequence but deleted in Clade-II. LAMP-1 assay primer regions show nucleotide differences in different regions from *Poxviridae* family members.



Supp Figure S2 Phylogenetic tree represents the evolutionary relationships of Monkeypox Clade-I with other *Orthopoxvirus* species. Vaccinia viruses and Cowpox virus are relatively close relatives to MPXV Clade-I, split by a genetic distance of 0.026 and 0.004, respectively. The genetic distance between MPXV Clade-II and Clade-I is 0.008. MPXV Clade-I separates Camelpox and Variola viruses by a genetic distance of 0.016 and 0.023, respectively, indicating more distantly related relationships.

Supp Table S1 Orthologous gene region mapping between MPXV-Clades and *Orthopoxvirus* family members and nucleotide locations of the region used in the phylogenic analysis

Virus Name	Genbank ID	NCBI ID	Position
Monkeypox_zaire 96-I-16 (Clade-I)	AF380138	NC_003310.1	18701-21151
MPXV-M5312_HM12_Rivers (Clade-II)	MT903340	NC_063383.1	18651-19150
Vaccinia virus WR (Western Reserve)	AY243312.	NC_006998.1	18433-20913
Vaccinia virus Copenhagen	M35027.1		21516-23966
Camelpox virus M-96 from Kazakhstan	AF438165.1	NC_003391.1	21230-23717
Cowpox virus Brighton Red	AF482758.2	NC_003663.2	34878-37351
Variola virus India-1967, ssp. major	X69198.1	NC_001611.1	11358-13755