



Supplementary Materials

Table S1. Primer sets used for whole genome sequencing of recent Bourbon virus (BRBV) strains from New York State.

| Primer | Upstream/downstream primer sequence (5'-3') | Target |
|---------------|---|----------------|
| BRBV-S6-20 | CGCTTCACAAGAGAAATGGC | Segment 6- M |
| BRBV-S6-868c | TGTAGAGGTTGTCTCTGATGAT | Segment 6- M |
| BRBV-S5-13 | CAGTTGTTCAACATGCAGTC | Segment 5- NP |
| BRBV-S5-1398c | CAGAGCCAGGCCTATGCCTCG | Segment 5- NP |
| BRBV-S4-12 | GCAGATTGTCCAAAGATGAAT | Segment 4- GP |
| BRBV-S4-1548c | TCTTAATAGACCCGCCTGCA | Segment 4- GP |
| BRBV-S3-13 | CAGTAATTGTCAATCATGGA | Segment 3- PA |
| BRBV-S3-1919c | CTACATATAAACATTTGGCA | Segment 3- PA |
| BRBV-S2-11 | AGCAGTTGTTAACCATGAATCT | Segment 2- PB1 |
| BRBV-S2-2152c | ACTAACCCAACCTCTCAACCAG | Segment 2- PB1 |
| BRBV-S1-24 | CATACGCACAGATGGAGGGCA | Segment 1- PB2 |
| BRBV-S1-1483c | TAGTTGGTGAGTTGCTGCATAC | Segment 1- PB2 |
| BRBV-S1-1238 | GACATTCTGTACTACACAGCATC | Segment 1- PB2 |
| BRBV-S1-2308c | CTACTTCCAGGAAGGTCCG | Segment 1- PB2 |

Table S2. Bourbon virus (BRBV) strains used in mutational analysis and phylogenetic trees including New York State BRBV strains, midwestern BRBV strains, and distantly related orthomyxoviruses Batken, Dhori, Oz, and Thogoto viruses.

| Strain Designation | Location | Year | Source | Accession Number |
|--------------------|---------------------|------|------------------------------|--|
| BRBV NY21-1814 | Suffolk County, NYS | 2021 | <i>A. americanum</i> pool | OQ513912, OQ513913, OQ513914, OQ513915, OQ513916, OQ513917 |
| BRBV NY21-2143 | Suffolk County, NYS | 2021 | <i>A. americanum</i> pool | OQ513918, OQ513919, OQ513920, OQ513921, OQ513922, OQ513923 |
| BRBV NY21-2666 | Suffolk County, NYS | 2021 | <i>A. americanum</i> pool | OQ513924, OQ513925, OQ513926, OQ513927, OQ513928, OQ513929 |
| BRBV Original | Bourbon County, MO | 2014 | Human | KP657750.3, KP657749.3, KU708255.1, KP657748.2, KU708254.2, KU708253.2 |
| BRBV STL | St. Louis, MO | 2017 | Human | MK453529.1, MK453528.1, MK453527.1, MK453526.1, MK453525.1, MK453524.1 |
| BRBV MO-2013-2499 | Gentry County, MO | 2013 | <i>A. americanum</i> pool | MH880287.1, MH880288.1, MH880289.1, MH880290.1, MH880291.1, MH880292.1 |
| Batken Virus | Kyrgyzstan | ? | <i>Hylamma spp.</i> | MT628416.1, MT628417.1, MT628418.1, MT628419.1, MT628420.1, MT628421.1 |
| Dhori Virus | ? | ? | ? | NC_034261.1, NC_034263.1, NC_034254.1, NC_034255.1, NC_034262.1, NC_034256.1 |
| Oz Virus | Ehime, Japan | 2013 | <i>A. testudinarium</i> pool | NC_040730.1, NC_040731.1, NC_040732.1, NC_040735.1, NC_040733.1, NC_040734.1 |
| Thogoto Virus | ? | ? | ? | NC_006508.1, NC_006495.1, NC_006496.1, NC_006506.1, NC_006507.1, NC_006504.1 |