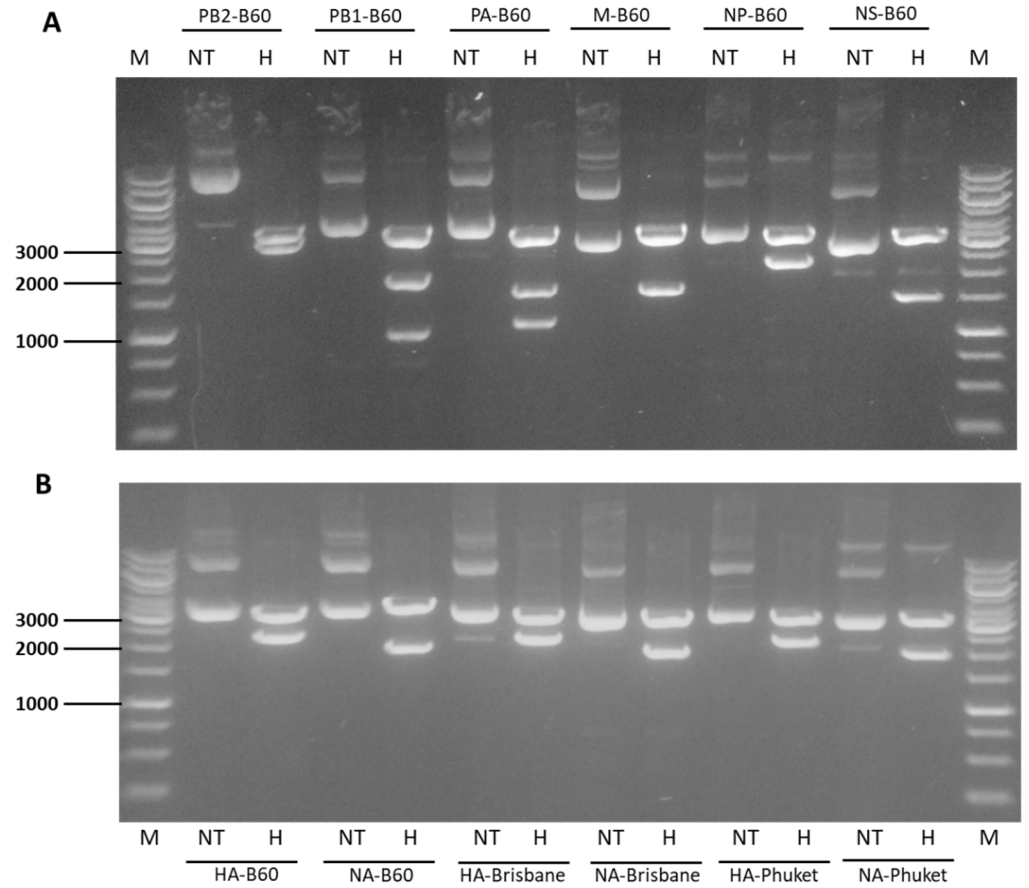


## Supplementary Materials

**Table S1.** Primer set used for RT-PCR amplification of the eight vRNAs of B/USSR60/69, HA and NA genes of B/Brisbane/60/2008 and B/Phuket/3037/2013; NA genes of 7+1 A/H2NBr and A/H2NPh.

Primer name	5'-Sequence-3' (*)
B-Uni_PB2-SapI_F	GATCGCTCTTCAGGG <b>AGCAGAAGCGGAGCGTTTTC</b> AAGATG
B-Uni_PB2-SapI_R	ACTGGCTCTTCTATT <b>AGTAGAAACACGAGCATT</b>
B-Uni_PB1-SapI_F	GATCGCTCTTCAGGG <b>AGCAGAAGCGGAGCCTTTA</b> AAGATG
B-Uni_PB1-SapI_R	ACTGGCTCTTCTATT <b>AGTAGAAACACGAGCCTT</b>
B-Uni_PA-SapI_F	GATCGCTCTTCAGGG <b>AGCAGAAGCGGTGCGTTTGA</b>
B/USSR_PA-BsmBI-1261R	TATTCGTCTCC <b>CAGGGCCCTTTTACTTGT</b> CAGAGTAC
B/USSR_PA-BsmBI-1283F	TATTCGTCTCT <b>CCTGGATCTACCAGAAATAGGGCC</b> CAGAC
B-Uni_PA-SapI_R	ACTGGCTCTTCTATT <b>AGTAGAAACACGTGCATT</b>
B-Uni_HA-SapI_F	GATCGCTCTTCAGGG <b>AGCAGAAGCAGAGCATT</b> TTTCTAATATC
B-Uni_HA-SapI_R	ACTGGCTCTTCTATT <b>AGTAGTAACAAGAGCATT</b> TTTTC
B-Uni_NP-SapI_F	GATCGCTCTTCAGGG <b>AGCAGAAGCACAGCATT</b> TTTCTTGTG
B/USSR-NP-1411R	<b>ACCCTCCGTCTCCACCTACTTCA</b>
B/USSR-NP-1433F	<b>TGAAGTAGGTGGAGACGGAGGGT</b>
B-Uni_NP-SapI_R	ACTGGCTCTTCTATT <b>AGTAGAAACAACAGCATT</b> TTTTTAC
B-Uni_NA-SapI_F	GATCGCTCTTCAGGG <b>AGCAGAAGCAGAGCA</b>
B-Uni_NA-SapI_R	ACTGGCTCTTCTATT <b>AGTAGTAACAAGAGCATT</b> TTT
B-Uni_M-SapI_F	GATCGCTCTTCAGGG <b>AGCAGAAGCACGCACTTTCTTAAAATG</b>
B-Uni_M-SapI_R	ACTGGCTCTTCTATT <b>AGTAGAAACAACGCACTTTT</b> TCCAG
B-Uni_NS-SapI_F	GATCGCTCTTCAGGG <b>AGCAGAAGCAGAGGATT</b> TGTTAGTC
B-Uni_NS-SapI_R	ACTGGCTCTTCTATT <b>AGTAGTAACAAGAGGATT</b> TTTAT
NA_B_H2_F	<b>AGCAAAAGCAGGAGTGAAAATGCTACCTTCAACTATAC</b>
NA_B_Bris_H2_H3_R	<b>AGTAGAAACAAGGAGTTTTTTCTAAAATTGCGAAAGCTTACAGAGC</b> <b>CATGTC</b>
NA_B_Phu_H2_H3_R	<b>AGTAGAAACAAGGAGTTTTTTCTAAAATTGCGAAAGCTTACAGAGT</b> <b>CATATTAACAC</b>

(\*) The sequences complementary to the influenza sequences are shown in bold. The 5'-ends have recognition sequences for the restriction endonuclease SapI or BsmBI. The design of the primers for PA and NP allowed the amplification of two fragments.



**Figure S1. (A).** RG plasmids carrying six internal protein segments of B/USSR/60/69 (B60) master donor virus, non-treated (NT) or hydrolyzed (H) with XbaI restriction enzyme. **(B).** RG plasmids carrying HA or NA segments of B/USSR/60/69 (B60) master donor virus or B/Brisbane/60/2008 or B/Phuket/3037/2013 wild-type viruses, non-treated (NT) or hydrolyzed (H) with XbaI restriction enzyme. M- DNA ladder.