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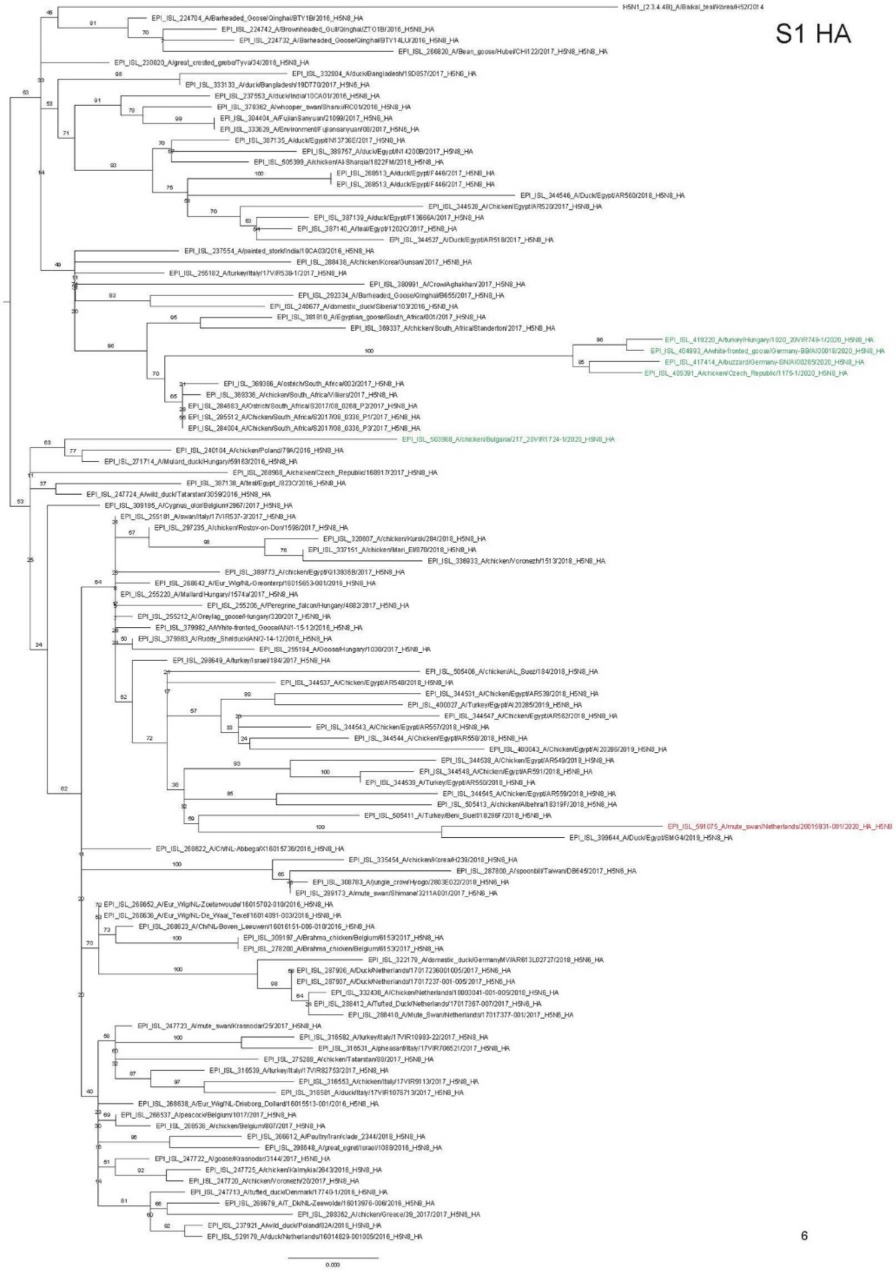
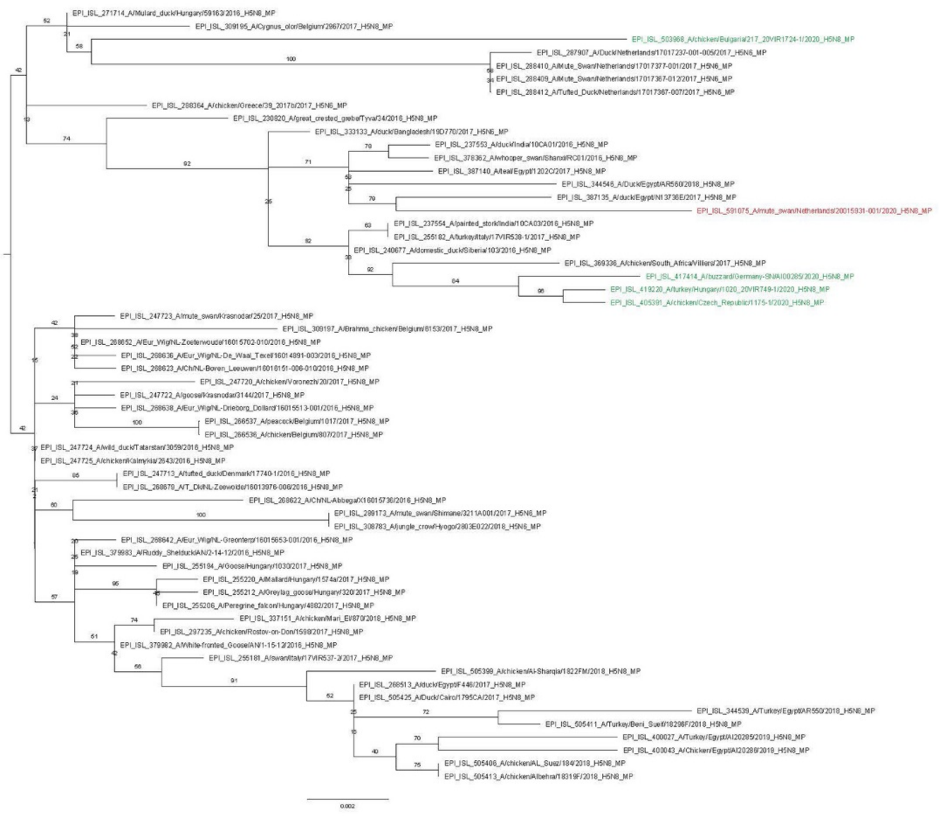
Incursion of Novel Highly Pathogenic Avian Influenza A(H5N8) Virus, the Netherlands, October 2020

Appendix

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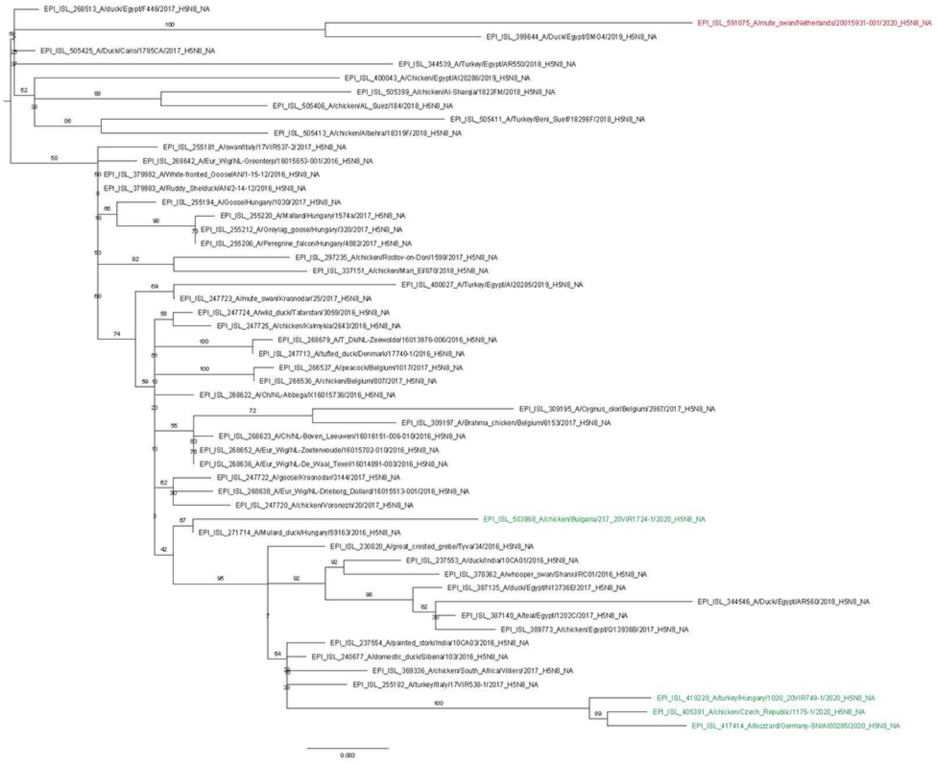
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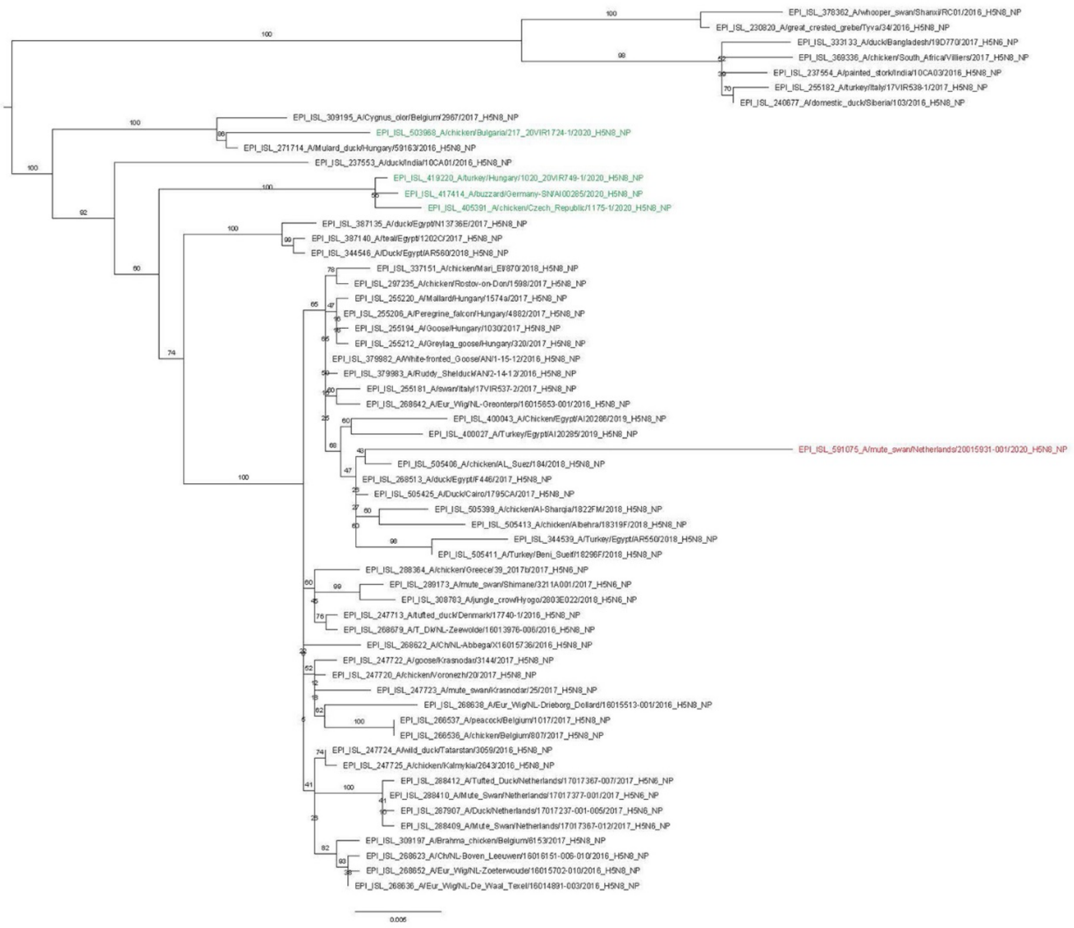
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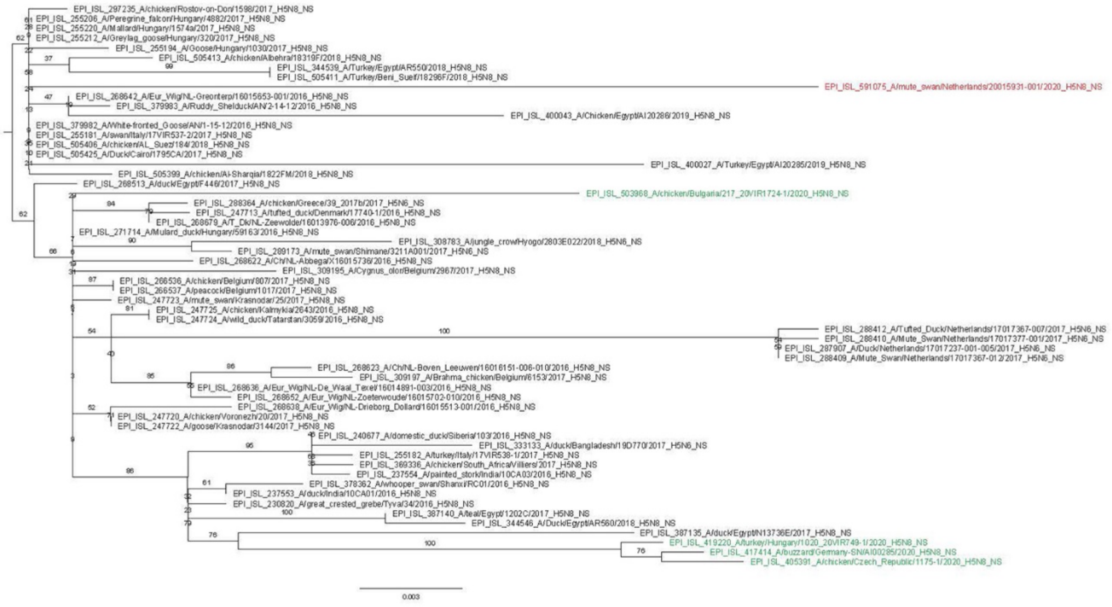
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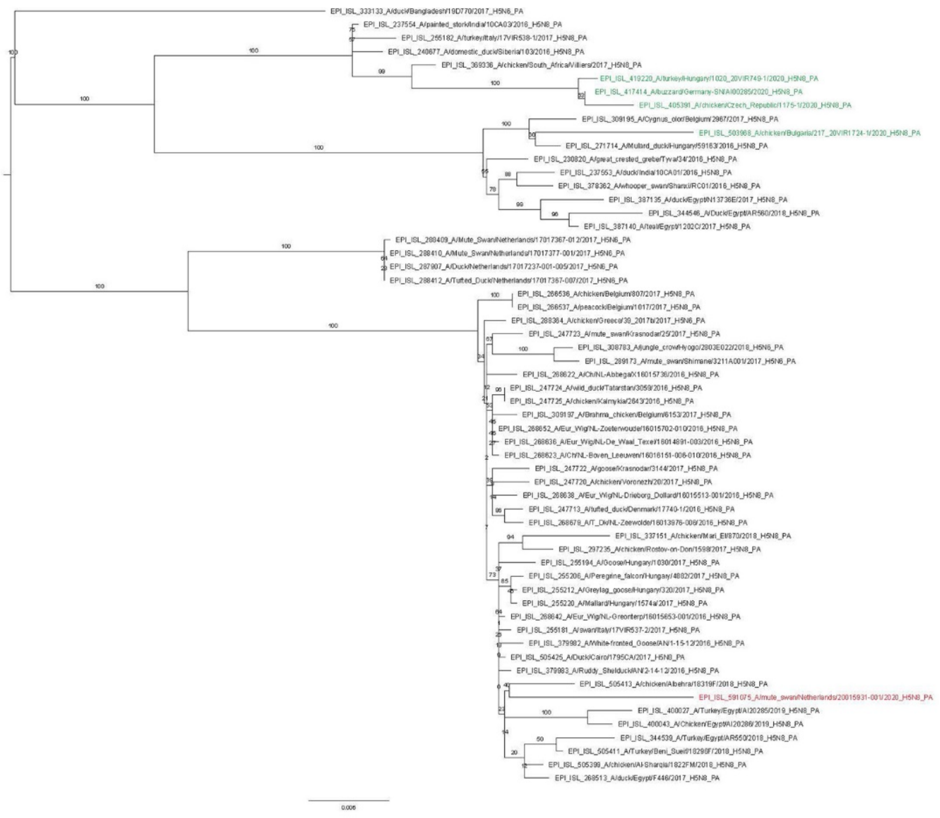
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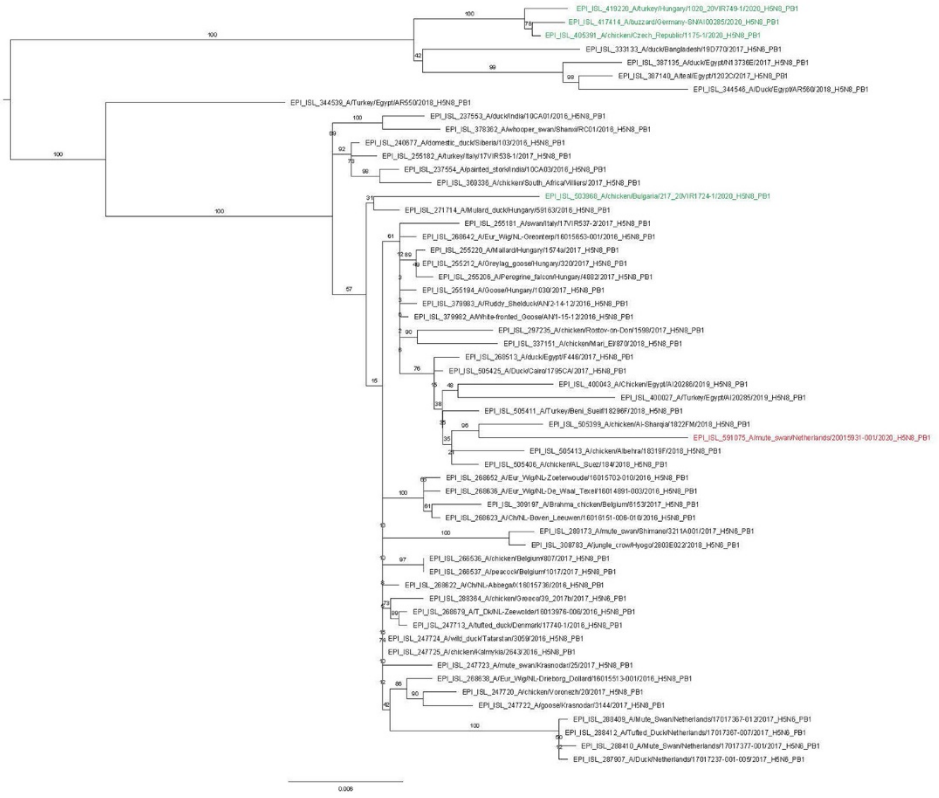
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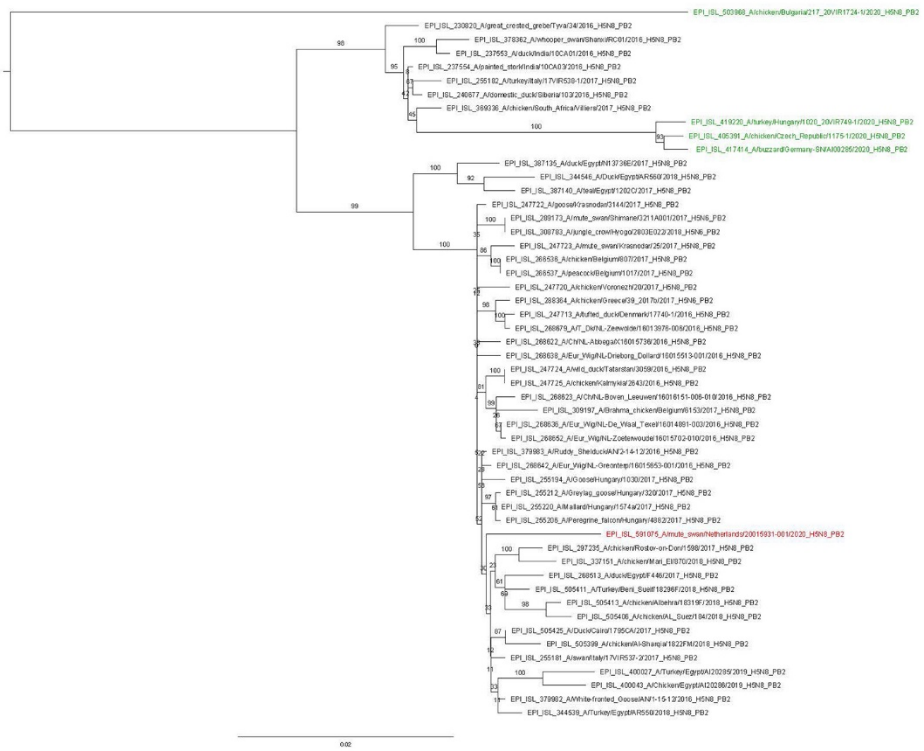
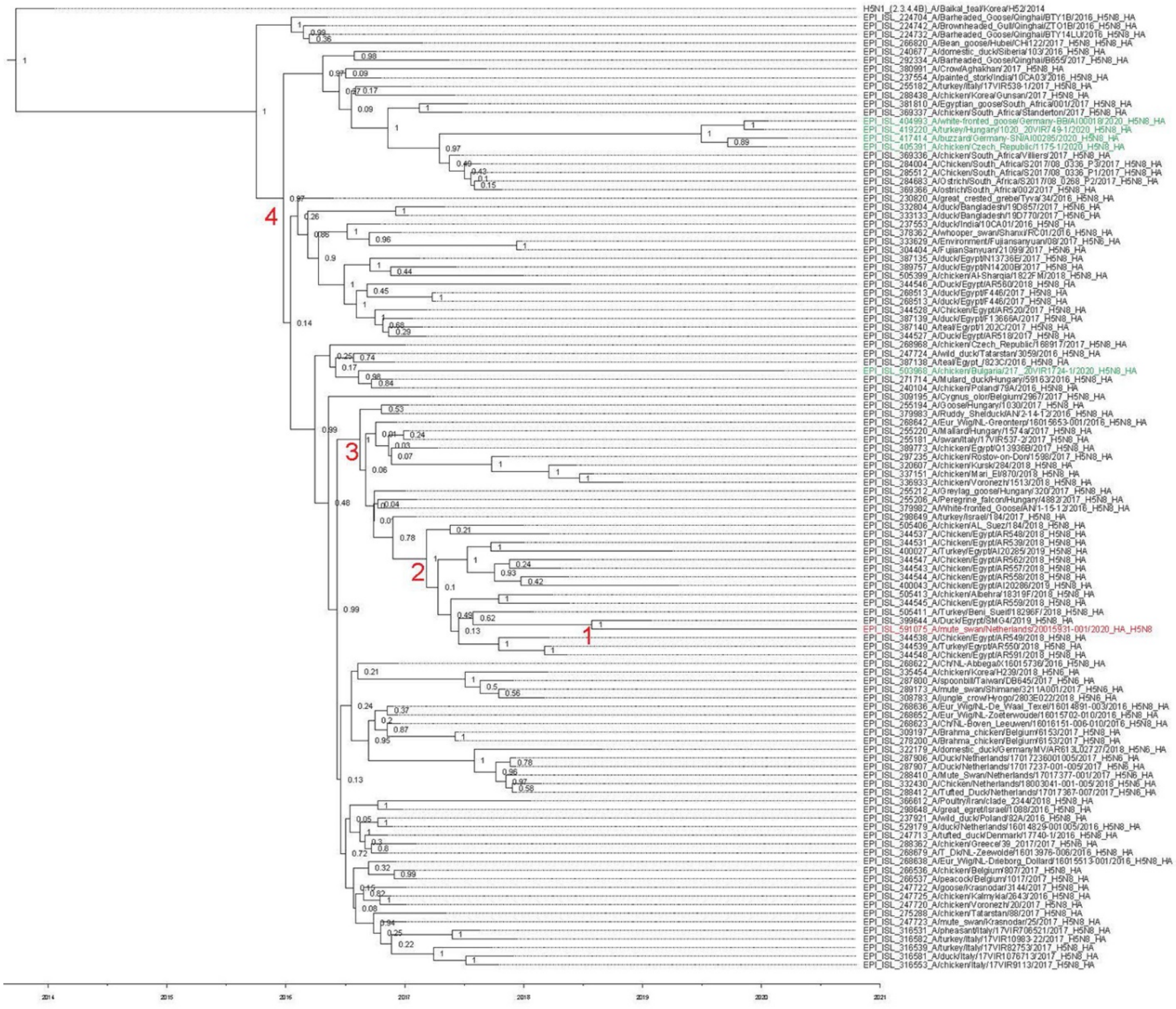


Appendix Figure 1. Related sequences were obtained from the GISAID EpiFlu database on October 21, 2020 (http://www.gisaid.org) (*1*) by using a BLAST search. For HA, additional sequences for H5 clade 2.3.4.4b were collected from the EpiFlu database and clustered by using the CD-HIT algorithm (*2*) and an identity setting of 0.985. Cluster representatives were used in the analysis of HA, in addition to the related sequences from the GISAID BLAST search. Sequences were aligned by using MAFFT v7.427 (*3*). Maximum-likelihood trees based on the general time-reversible model with a gamma-distributed variation of rates, and 100 bootstraps were generated by using RAxML v8.2.12 (*4*). The GISAID accession numbers of the viruses are shown in the trees (Appendix Table). The H5N8 virus isolated in the Netherlands in 2020 is marked in red, the H5N8 viruses isolated in Eastern-Europe, Germany and Bulgaria in 2020 are marked in green. Scale bars indicate nucleotide substitutions per site. HA, hemagglutinin; MP, matrix protein; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural protein; PA, polymerase acidic, PB1, polymerase basic 1; PB2, polymerase basic 2.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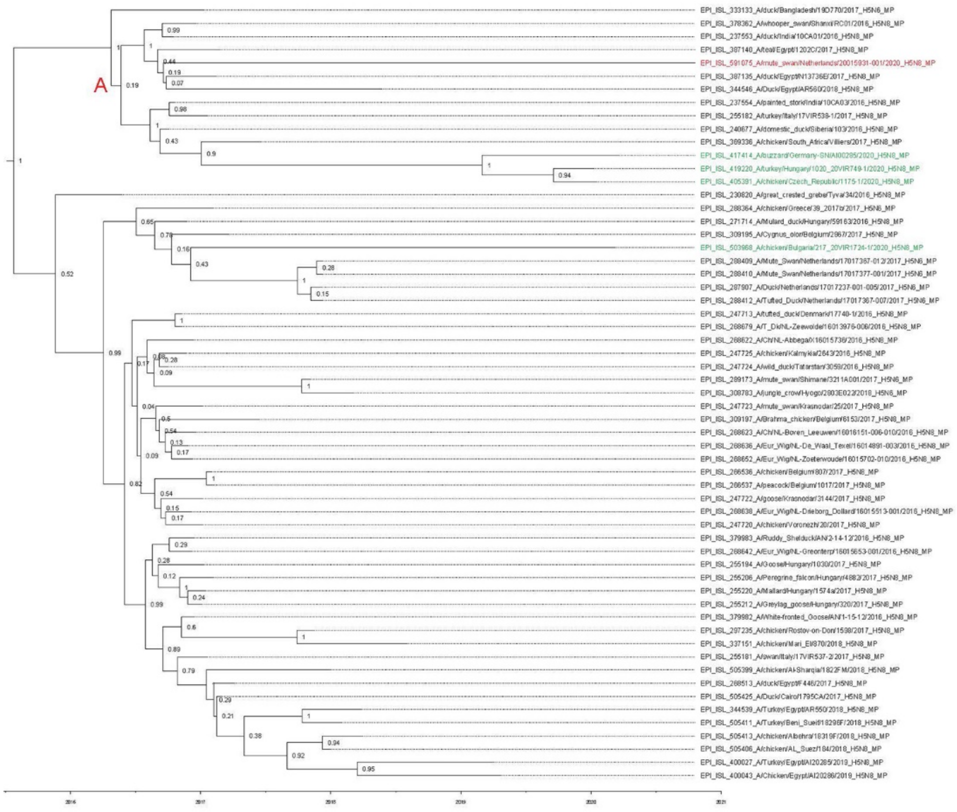
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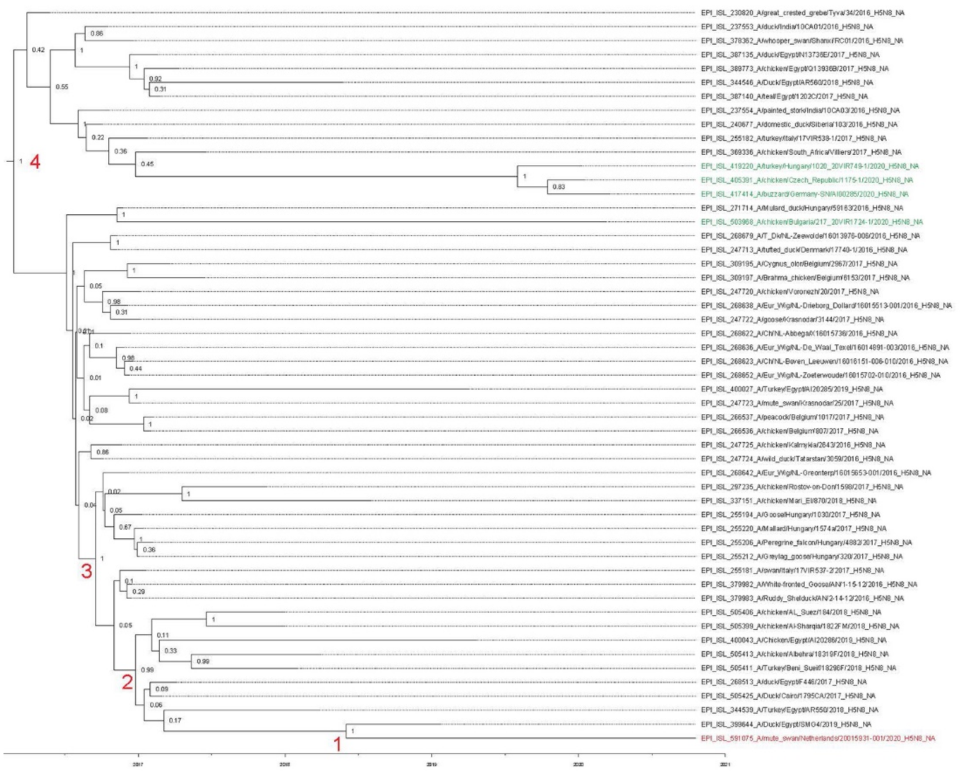
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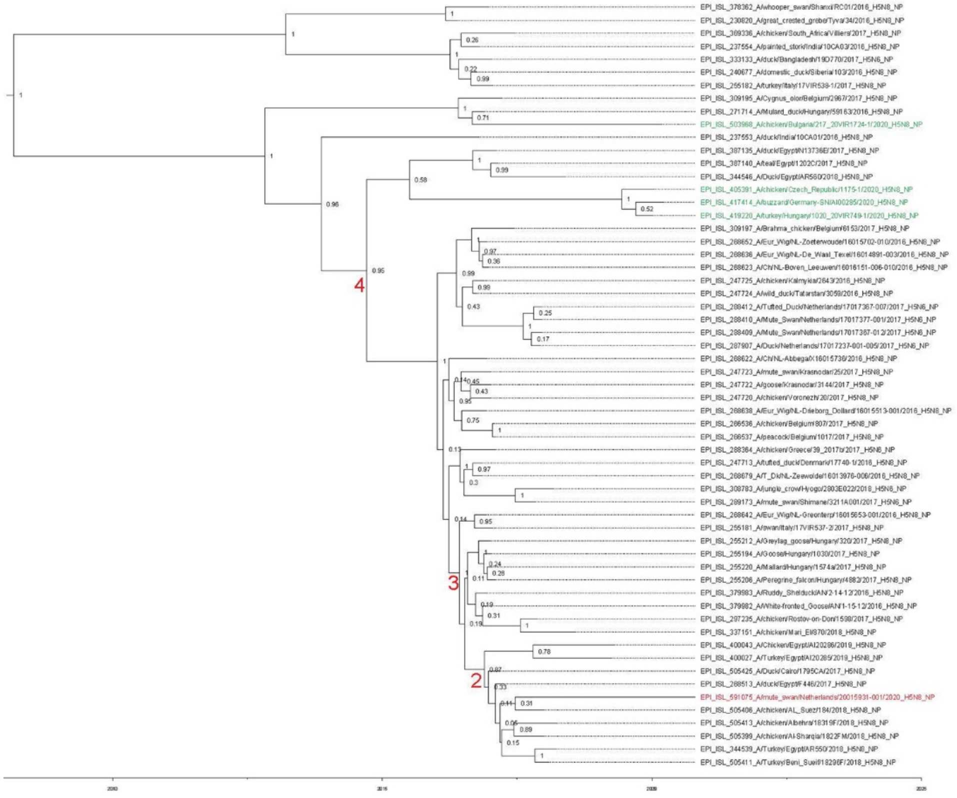
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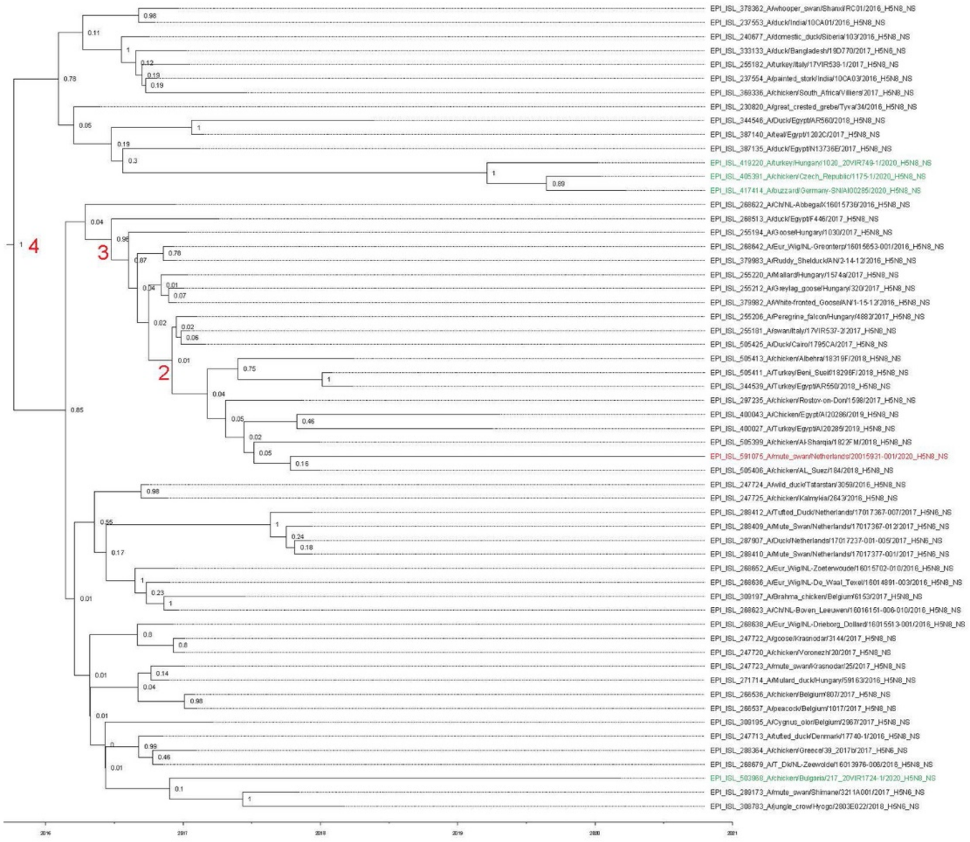
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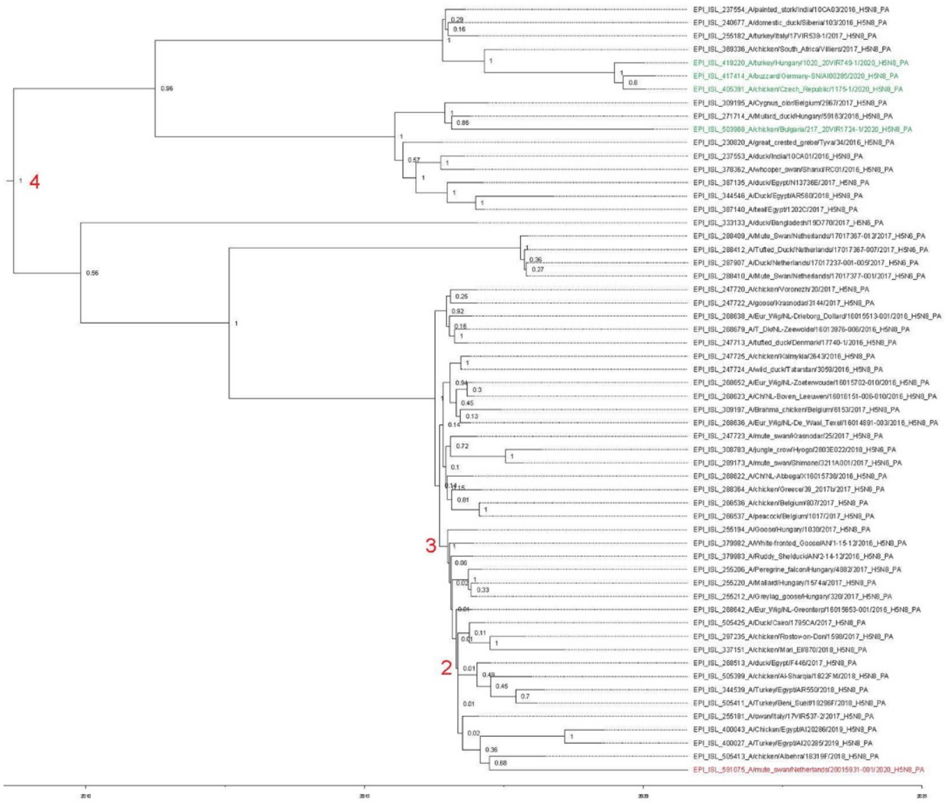
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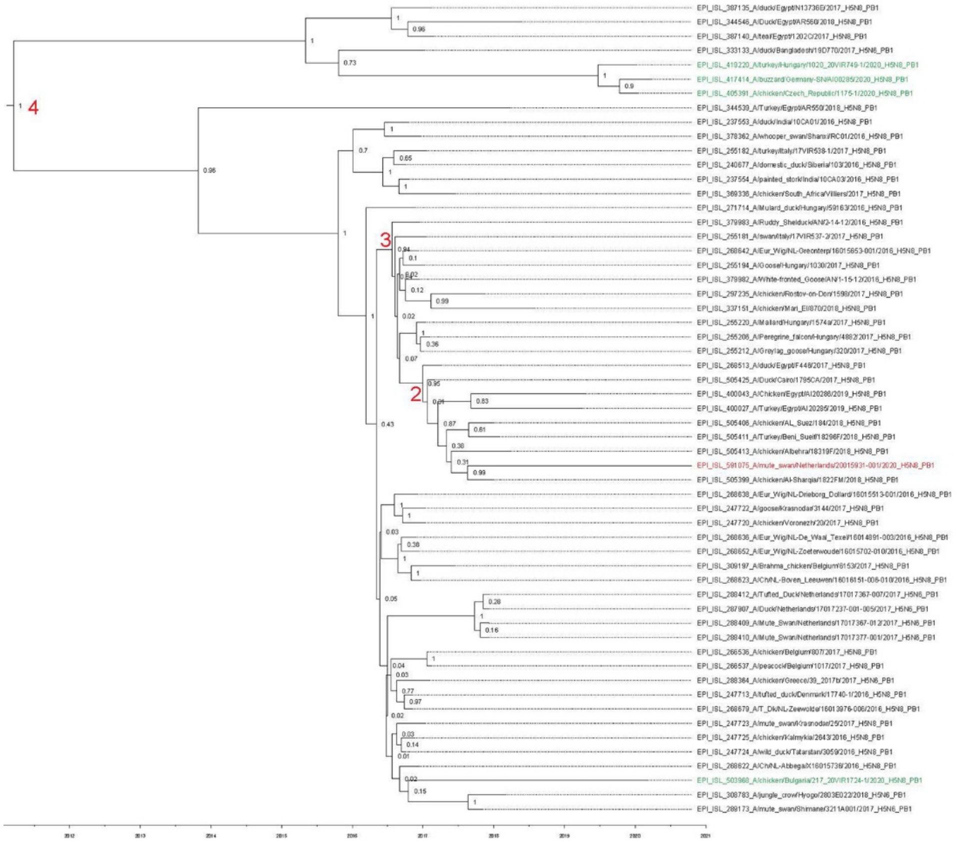
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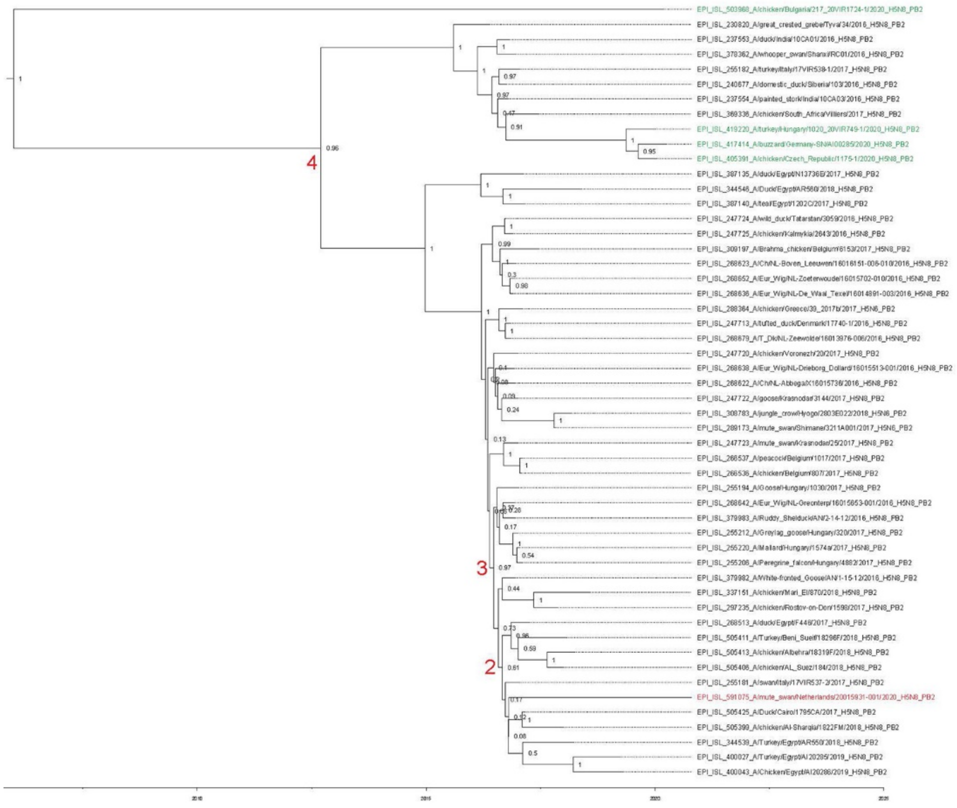

Appendix Figure 2. Molecular dating was performed for the all gene segments. Datasets of maximum-likelihood tree analysis (Appendix Figure1) were used for time-scaled phylogenies, which were reconstructed by using a Bayesian Markov chain Monte Carlo framework implemented in the BEAST software package v 1.10.2 (*5*). Analysis was conducted by using the SRD06 nucleotide substitution model, the Bayesian Skyline coalescent model, and an uncorrelated log normal relaxed molecular clock. Markov chain Monte Carlo runs of 1 x 10^8 states sampling each 1 x 10^4 steps were run to obtain an effective sample size >200. Maximum clade credibility trees were reconstructed with 10% burn-in, and the posterior distribution of relevant parameters were assessed in FigTree v 1.4.4 (*6*). The time to the most recent common ancestor for the numbered nodes is listed in the Table, as is the credible interval and posterior value. For the MP segment, the letter A was used in this figure and the Table to denote the relationship with the viruses found in eastern Europe and Germany during 2020. Because the MP segment was probably introduced by reassortment, this node is not similar to node 4 for the other segments. GISAID accession numbers are shown in the trees (Appendix Table). H5N8 virus isolated in the Netherlands during 2020 is indicated in red; and viruses isolated in eastern Europe, Germany, and Bulgaria during 2020 are indicated in green. Scale bars indicate time intervals. HA, hemagglutinin; MP, matrix protein; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural protein; PA, polymerase acidic, PB1, polymerase basic 1; PB2, polymerase basic 2; 4, node 4.

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