

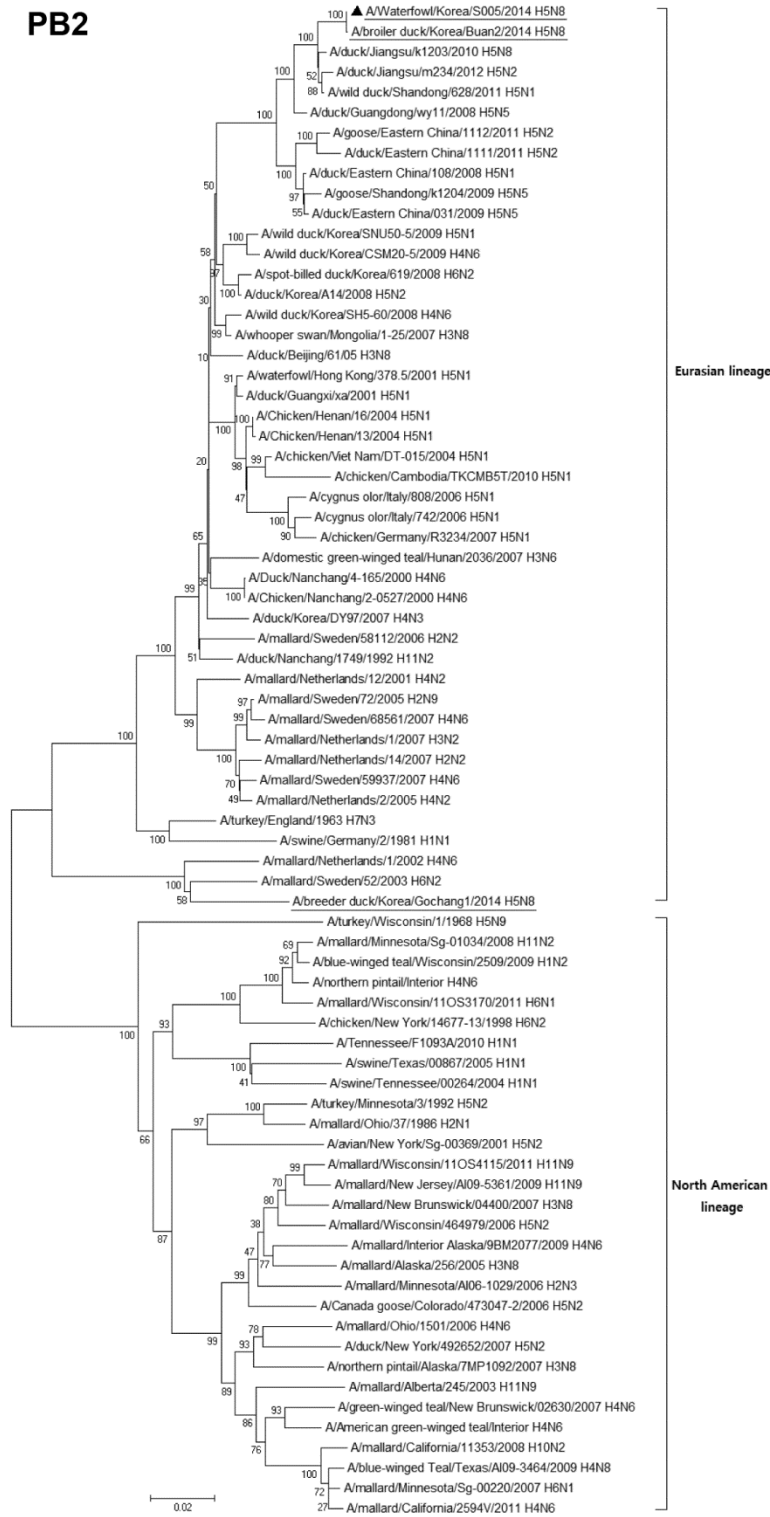
Highly Pathogenic Avian Influenza A(H5N8) Virus from Waterfowl, South Korea, 2014

Technical Appendix

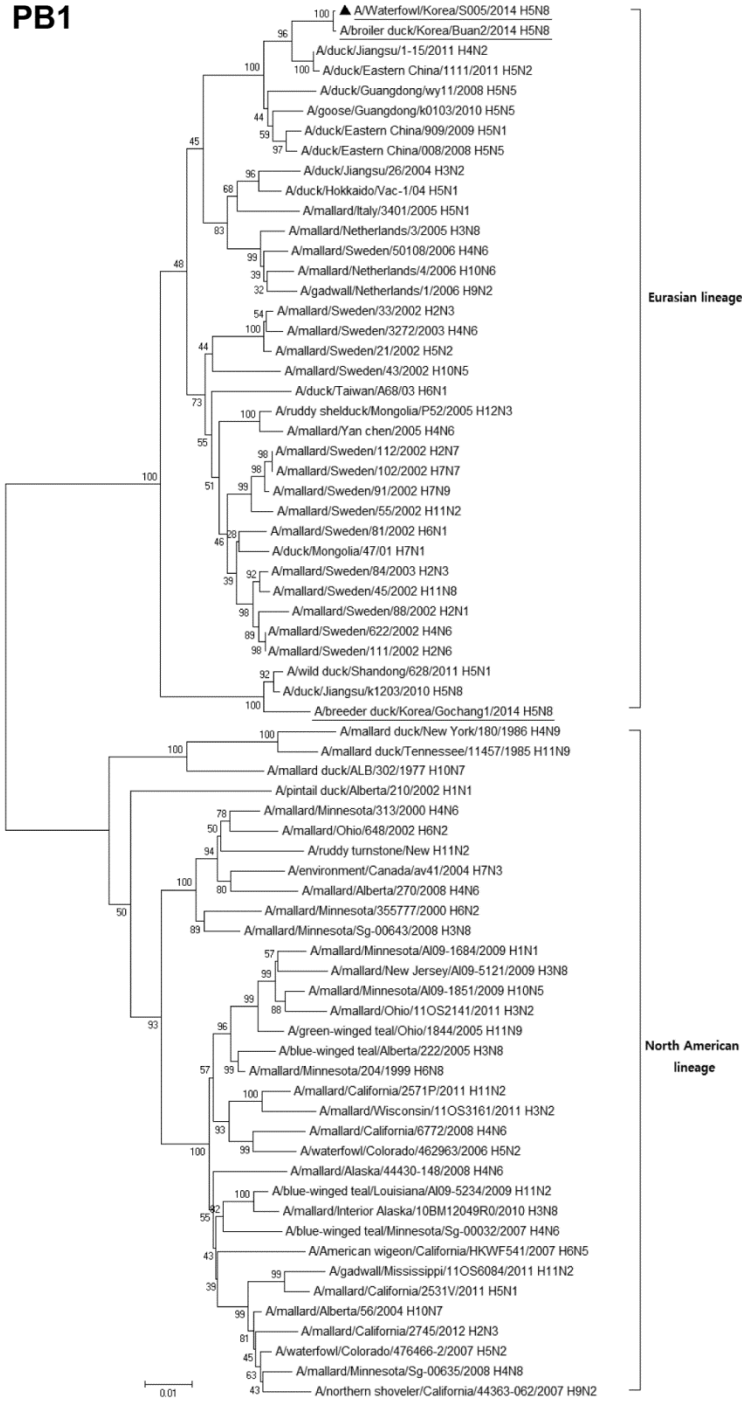
Technical Appendix Figure 1 (following pages). Phylogenetic analysis of PB2, PB1, PA, HA, NP, NA, M, and NS genes of influenza virus strain A/waterfowl/Korea/S005/2014 (H5N8) (indicated by triangles). The trees were constructed using the neighbor-joining method in MEGA5 (<http://www.megasoftware.net>) with 1,000 bootstrap replicates. Scale bars indicate nucleotide substitutions per site. The HA was rooted to A/Goose/Guangdong/1/1996. The clade of HA gene was determined by BLAST search (<http://www.fludb.org/brc/h5n1Classifier.spg?method=ShowCleanInputPage&decorator=influenza>).

Underlines indicate recent H5N8 isolates. PB, polymerase basic subunit; PA, polymerase acidic subunit; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix; NS, nonstructural.

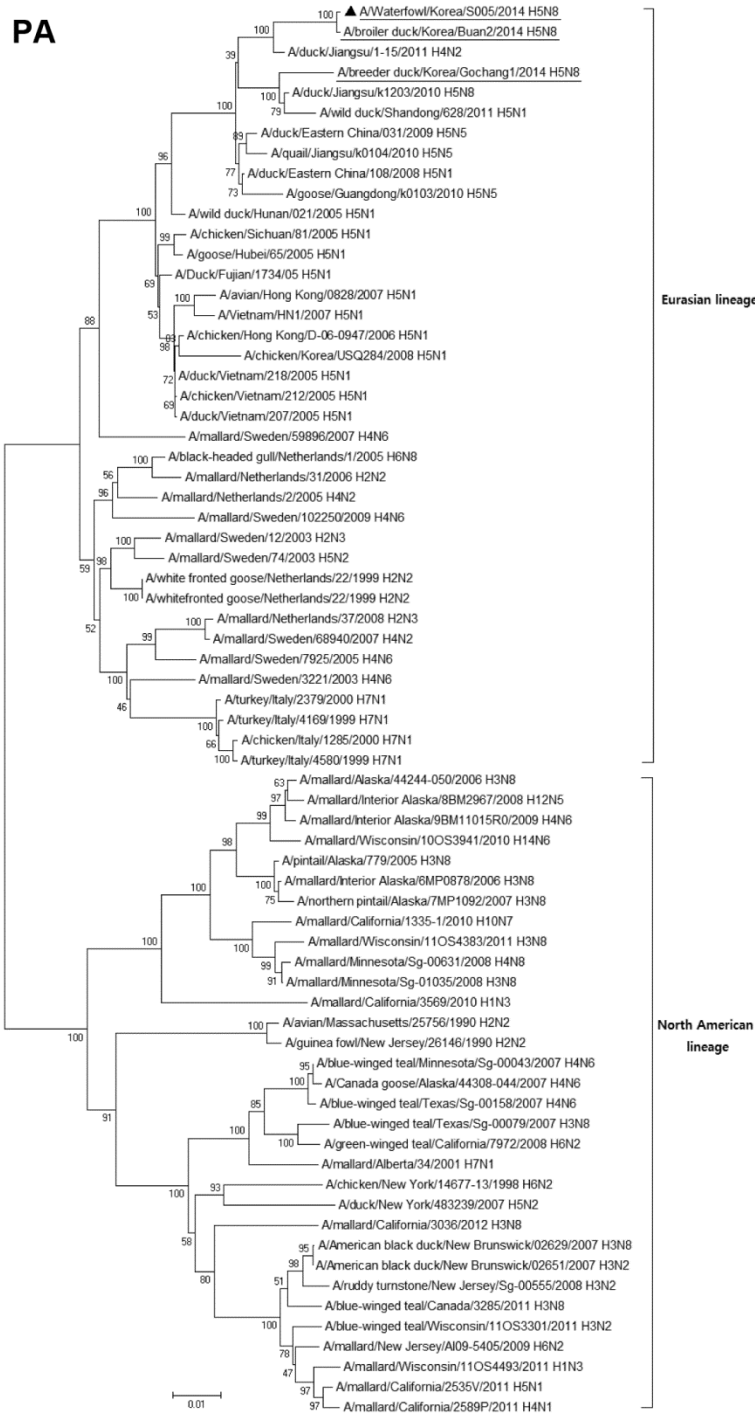
PB2



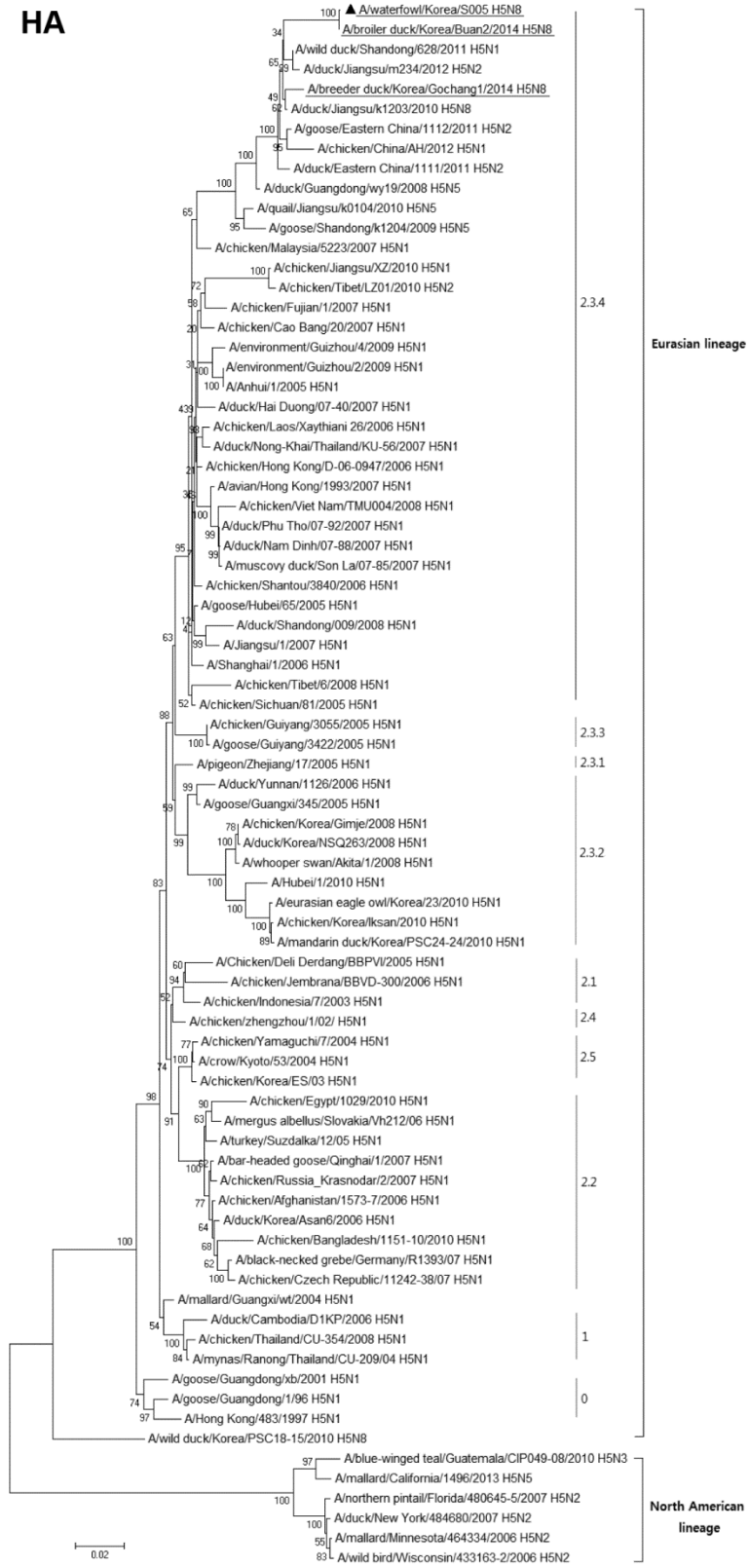
PB1



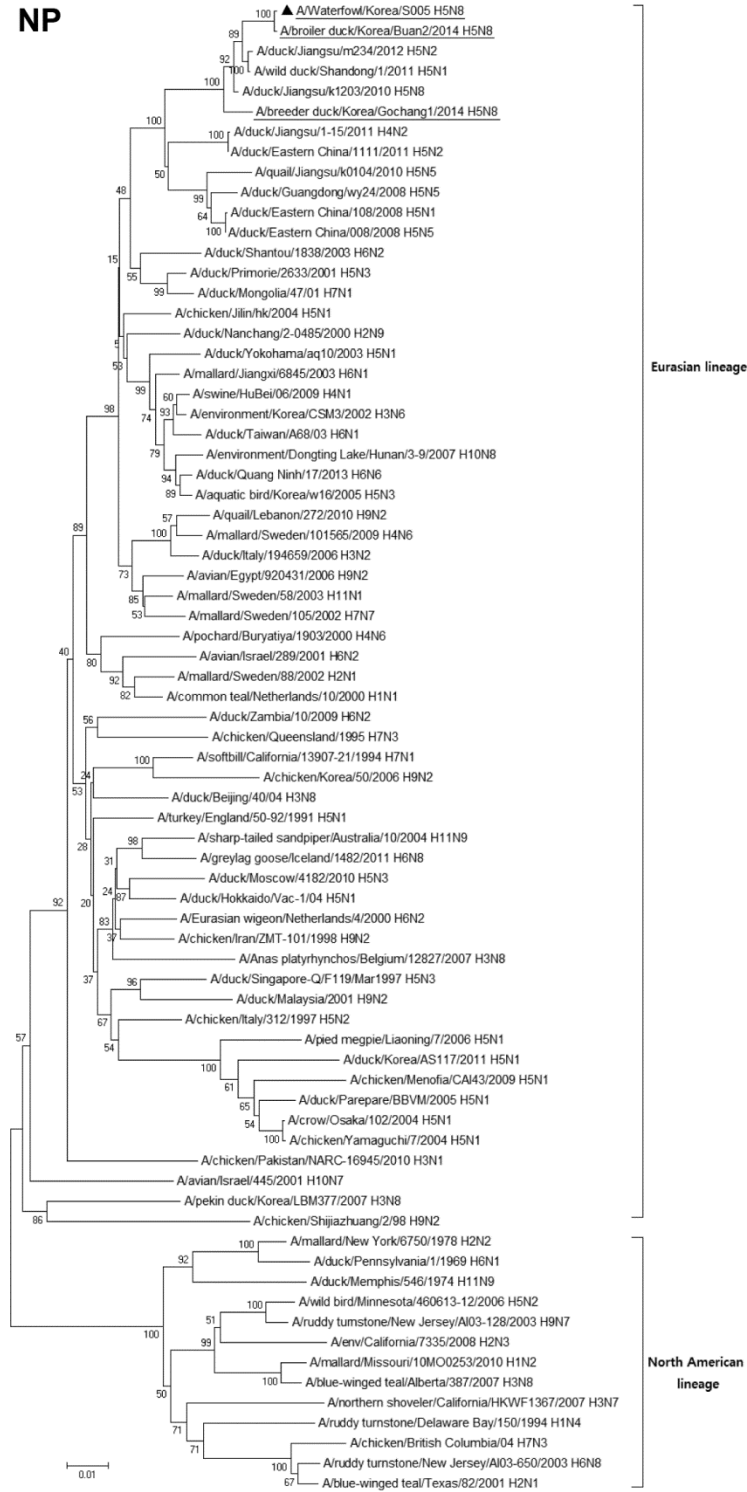
PA



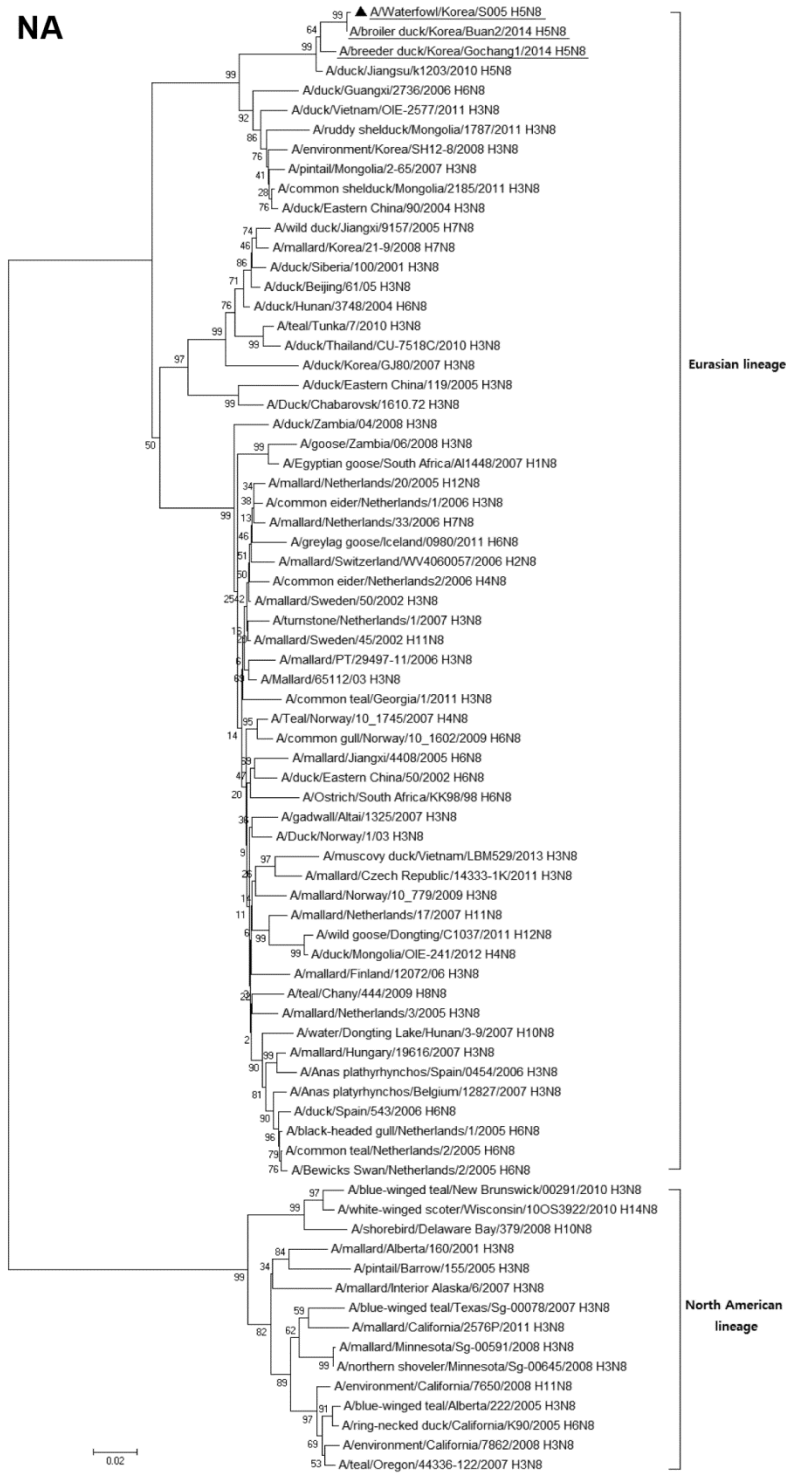
HA



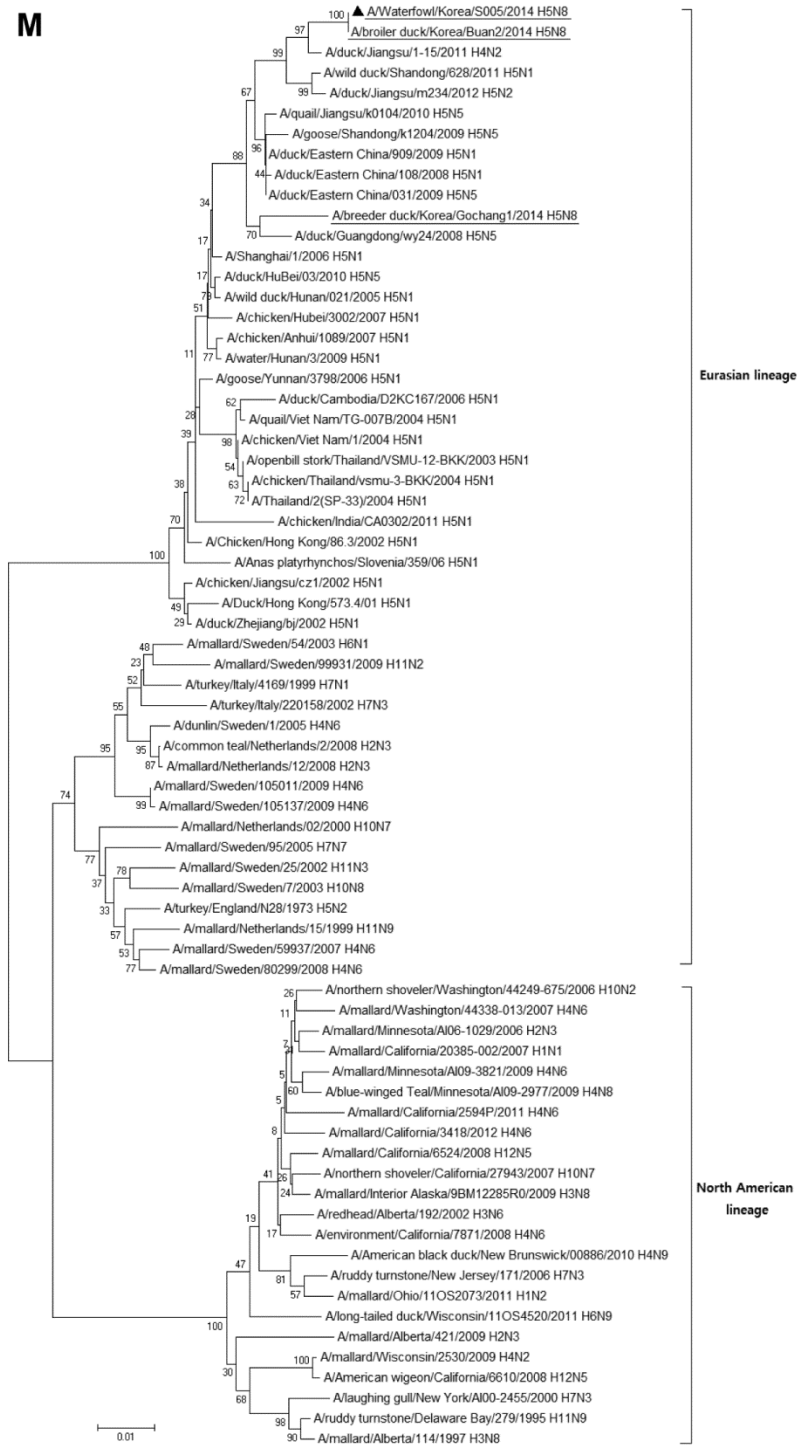
NP



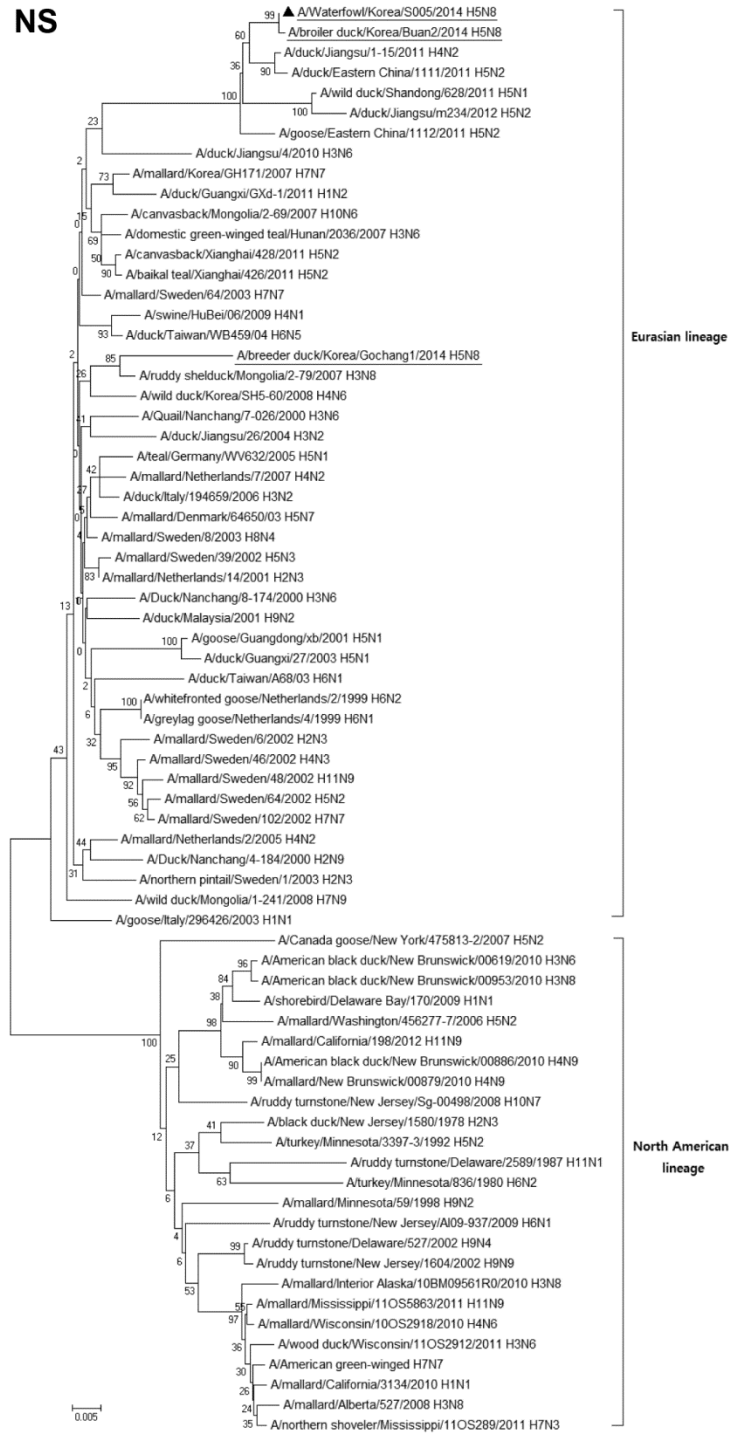
NA

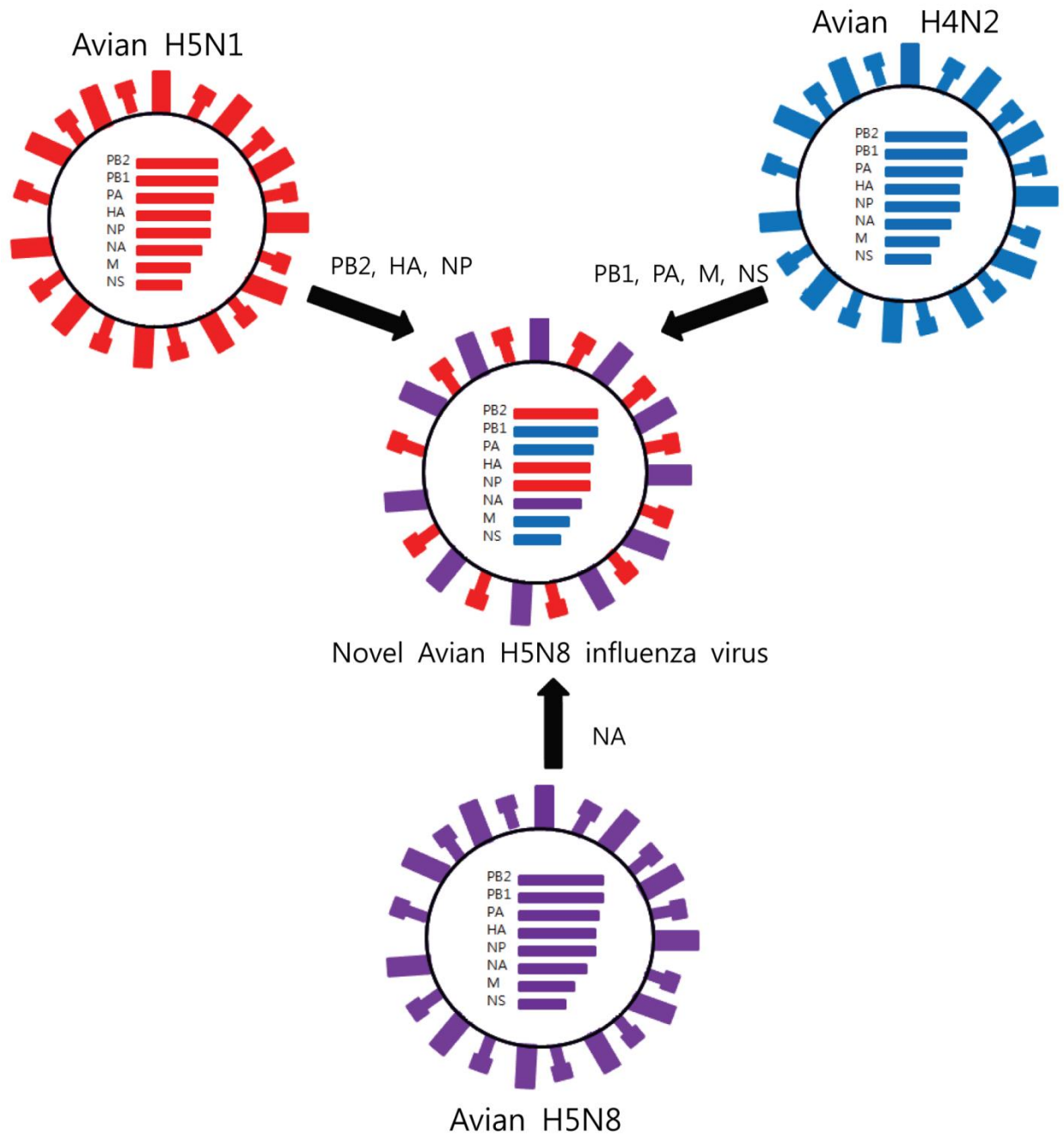


M



NS





Technical Appendix Figure 2. Schematic diagram of influenza virus strain A/waterfowl/Korea/S005/2014 (H5N8). Novel highly pathogenic avian influenza virus is likely to be created by genes from 3 avian influenza viruses. PB, polymerase basic subunit; PA, polymerase acidic subunit; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix; NS, nonstructural.

Technical Appendix Table. Identification of amino acids of influenza virus strain A/waterfowl/Korea/S005/2014 (H5N8) involved in binding to human-type influenza receptor, enhancing antiviral drugs, and causing pathogenesis in poultry and mammals

Viral protein*	Amino acid position	A/waterfowl/Korea/S005/2014 (H5N8)†	Comments
PB2	627	E	E627K: adaptation to mammalian host
HA	138 (H3 numbering)	A	S138A: Increased binding to human-type influenza receptor
	160 (H3 numbering)	A	T160A: N-glycosylation loss and increased binding to human-type influenza receptor
	226 (H3 numbering)	Q	Q226L: Increased binding to human-type influenza receptor
	228 (H3 numbering)	G	G228S: Increased binding to human-type influenza receptor
	339-348	RE RRRK R/GLF	Polybasic amino acid insertion: high pathogenesis in poultry and mammals
NA	69-72 (N9 numbering)	No deletion	Deletion of amino acids 69-73: Increased pathogenesis in mice
	292 (N2 numbering)	R	R292K: Resistance to oseltamivir and zanamivir
M1	30	D	N30D: Increased pathogenesis in mice
	215	A	T215A: Increased pathogenesis in mice
M2	31	N	S31N: Resistance to amantadine and rimantadine
NS1	42	S	P42S: Increased pathogenesis in mice
	218-230	No truncation	Lack of PDZ domain binding motif: reduced pathogenesis in mice

*PB, polymerase basic subunit; PA, polymerase acidic subunit; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix; NS, nonstructural.

†A, alanine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; K, lysine; L, leucine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine.