

Novel Reassortant Influenza A(H1N2) Virus Derived from A(H1N1)pdm09 Virus Isolated from Swine, Japan, 2012

Technical Appendix

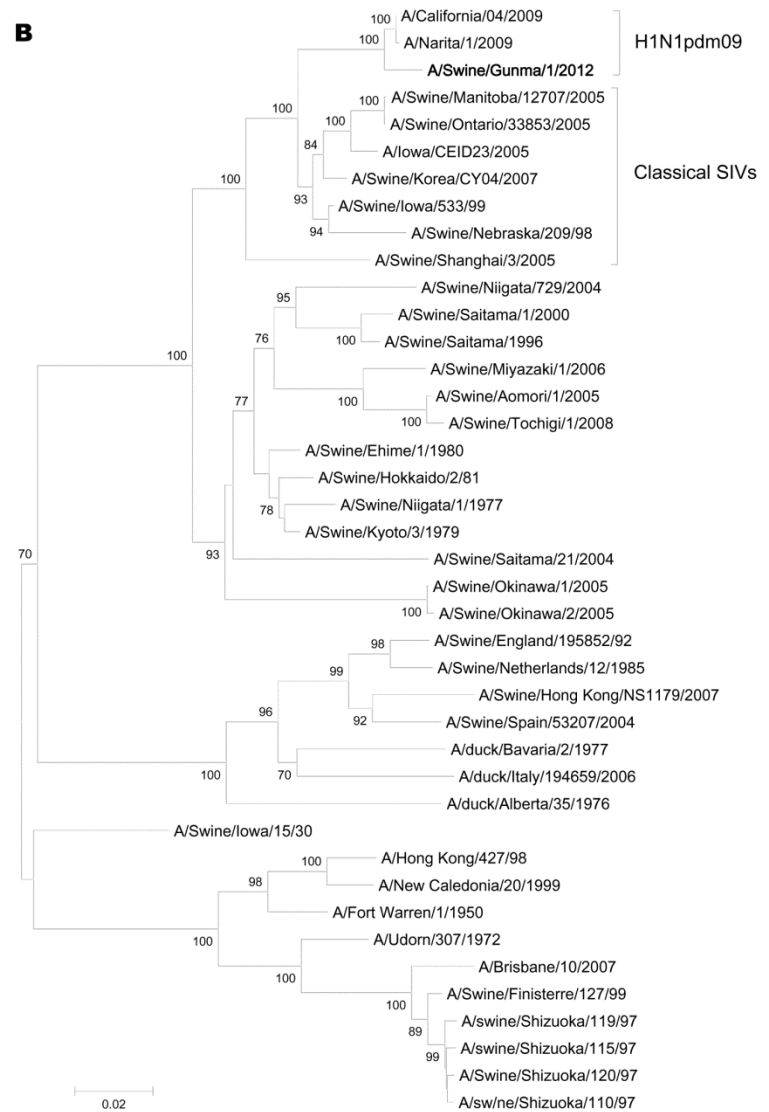
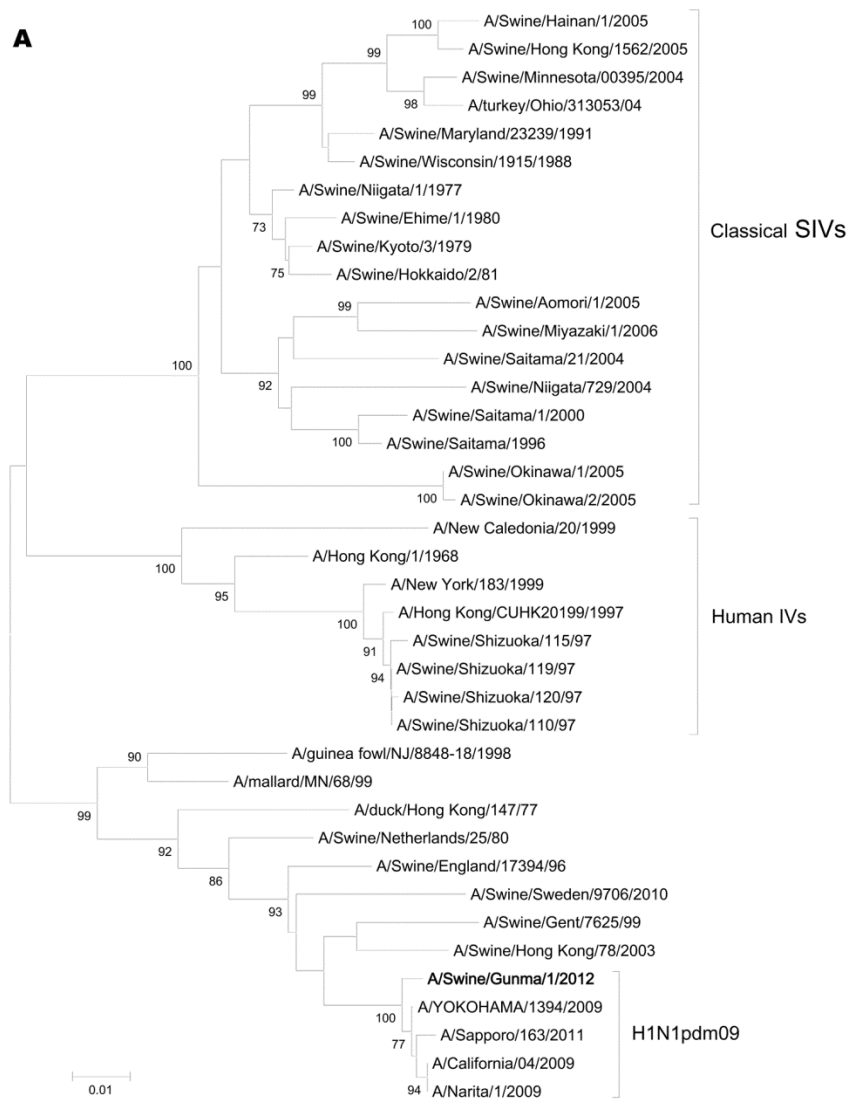
Genome Amplification, Sequencing, and Phylogeny of Novel Reassortant Influenza A(H1N2) Virus

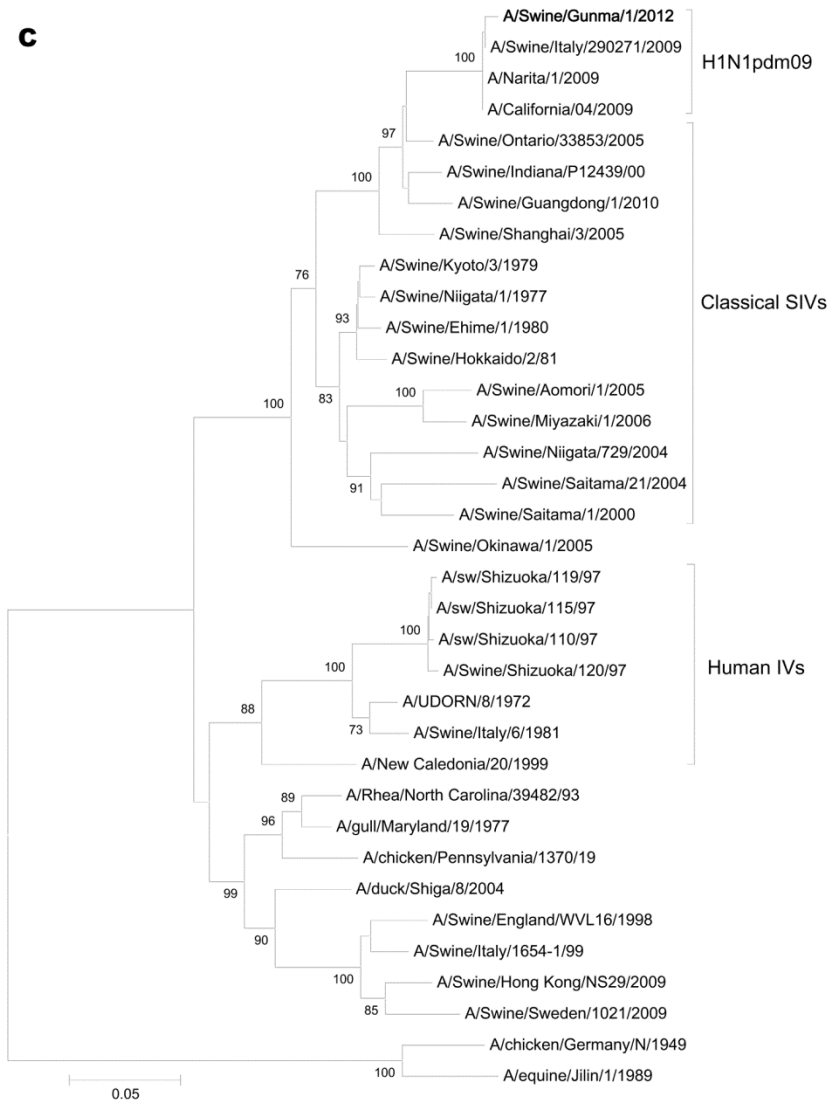
Technical Appendix Table. Primers for amplification and sequencing of the full genome of A/swine/Gunma/1/2012, a novel swine influenza A(H1N2) virus strain, Japan, 2012

Gene	Primer	Sequence (5'→3')
Amplification		
HA	5+HA-1	CAGGGAGCAAAAGCAGGGG
	11+NS-890R	CGTCTCGTATTAGTAGAAACAAGGGTGT
NA	7+NA-1	CTCAGGGAGCAAAAGCAGGAGT
	12+NA-1413R	TGGTCTCGTATTAGTAGAAACAAGGAGTTTTT
PB2	PB2-F	AGCAAAAGCAGGTCAAWTATTC
	PB2-R	AGTAGAAACAAGGTCGTTTTTAAA
PB1	PB1-1	AGCAAAAGCAGGCAAACCATT
	PB1-R	AGTAGAAACAAGGCATTTTTTCA
PA	PA-1-26F	AGCRAAAGCAGGTAAGTATGATYCRAAAT
	PA-2228R	AGTAGAAACAAGGTAAGTTTTTGGACAG
NP	NP-F	AGCAAAAGCAGGGTARATAATCATCTC
	Bm-NP-1565R	AGTAGAAACAAGGGTATTTTT
MP	MP-F	AGCAAAAGCAGGTAGATRTTKAAAG
	MP-R	AGTAGAAACAAGGTAGTTTTTTACTC
NS	NS-F	AGCAAAAGCAGGGTGACAAA
	NS-890R	AGTAGAAACAAGGGTGTTTTTTATCA
Sequencing		
HA	5+HA-1	CAGGGAGCAAAAGCAGGGG
	swineH1-56-76F	CATTATGTATAGGTTATCATG
	swineH1-366-385F	GAGCTCAGTGTCATCATTTG
	swineH1-596-578R	GGATGGTGAATGCCCCATA
	swineH1-768-788F	AATAACATTCGAAGCAACTGG
	swineH1-1106-1087R	TGATAACCGTACCATCCATC
H1N1pdmHA-1300-1320F	GACATTTGGACTTACAATGCC	
NA	7+NA-1	CTCAGGGAGCAAAAGCAGGAGT
	N2-548R	TGACAACTTGAGCTGGACCA
	N2-1062R	AAGGCCAGCCTTTCACYCC
	N2-902F	TGGAAGGGCTCYAATAGGCC
	12+NA-1413R	TGGTCTCGTATTAGTAGAAACAAGGAGTTTTT
PB2	PB2-F	AGCAAAAGCAGGTCAAWTATTC
	PB2-599R	ATGTATGCCACCATYAAGGG
	H1N1pdmPB2-280-300R	TTCCACCATGTTACGGCCAGA
	H5PB2-1241R	TATCATGCAATCCTCCTGTG
	H5PB2-1071F	CCTCCAAACATTGAAAATAAGAG
	PB2-R	AGTAGAAACAAGGTCGTTTTTAAA
H1N1pdmPB2-1650-1670F	CAATGGATAATCAGGAACTG	
PB1	H1N1pdmPB1-290-310R	CAAATATTCCTGGGTGGGATT
	PB1-1	AGCAAAAGCAGGCAAACCATT
	PB1-1240R	AACATGCCCATCATCATTCC
	PB1-1334R	GATTGGAGTCGATCCCACCA
	PB1-1121F	CACAAATACCAGCAGAAATGC
PB1-R	AGTAGAAACAAGGCATTTTTTCA	

Gene	Primer	Sequence (5'→3')
	PB1-1651F	ACAGCTCAGATGGCTCTTCA
PA	PA-230-249R	TTCAATTATCTCAAATCGGT
	H5PA-583R	TGACGAAAGGAATCCCATAG
	H5PA-686R	TTCAAGGCTGGAGAAGTTCCG
	PA-940Rv2	TCTTCATGCATTTGATTGCATC
	PA-1-26F	AGCRAAAGCAGGTAATGATYCRAAT
	H5PA-583F	CTATGGGATTCTTTTCGTC
	H5PA-1818R	TCTCTTTGACAGAAGACTCG
	PA-940F	GATGCAATCAAATGCATGAAGA
	H5PA-1147F	GCACCAGAGAAAGTAGACTT
	PA-2228R	AGTAGAAAACAAGGTAATTTTTGGACAG
	H5PA-1619F	TGGAGCCACACAAGTGGGAA
H5PA-F1792	GCCGAGTCTTCTGTCAAAGA	
NP	H1N1pdmNP-500-520R	TGGGAAGTGTTGACCCTTGCA
	NP-F	AGCAAAAGCAGGGTARATAATCACTC
	H5NP-1075R	GTCCCTCTGATGAAACTTGA
	H5NP-451F	CTGATGATATGGCATTCCAA
	NP-1565R	AGTAGAAAACAAGGGTATTTTTCTT
	H5NP-451F	CTGATGATATGGCATTCCAA
H1N1pdmNP-1000-1020F	CACTCTGCTGCATTGAAGAT	
MP	MP-501r2	CGATGCTGTGAATCTGCAAT
	MP-R	AGTAGAAAACAAGGTAGTTTTTACTC
	MP-F	AGCAAAAGCAGGTAGATRTTKAAAG
	H5MP-501F	ATTGCAGATTCACAGCATCG
NS	NS-F	AGCAAAAGCAGGGTGACAAA
	H5NS-535R	GTGTTATCATTCCATTCAAGTCC
	NS-890R	AGTAGAAAACAAGGTGTTTTTATCA
	H1N1pdmNS-450-470F	CACTGAGGAGGGAGCAATAGT

Technical Appendix Figure (following pages). Phylogenetic analysis of the A/swine/Gunma/1/2012 strain of the influenza A(H1N1)pdm09 virus isolated from swine. Phylogenetic trees based on A) the matrix protein (MP), B) nucleoprotein (NP), C) nonstructural protein (NS), D) polymerase acid (PA), E) polymerase basic 1 (PB1), and F) polymerase basic 2 (PB2) genes were constructed by using the neighbor joining method. Bold text represents novel H1N2 swine influenza virus (SIV) isolated in Japan during January 2012. Numbers along branches represent estimates made with 1,000 bootstrap replications to demonstrate the reliability of the trees. Scale bars represent nucleotide substitutions by site.



C**D**