

Table S3. List of All Non-Synonymous and Synonymous Mutations Identified During Sequential Transmission Experiments with A/Anhui/1/2013 (H7N9)

Gene Segment	Nucleotide Change	Amino Acid Change Relative to Inoculum [(for HA, (H3 numbering)]	Nucleotide Frequencing																		
			Virus Inoculum	Trio 1: DI 7090 - no transmission to RC1			Trio 2: DI 7094 - no transmission to RC1			Trio 3: DI 7097 - no transmission to RC1			Trio 4: DI 6811 -> RC1 7077 -> RC2 7092			Trio 5: DI ARC1196 -> RC1 1197 -> RC2 7096			Trio 6: DI 7078 -> RC1 7080 - no transmission to		
				DI	RC1	RC2	DI	RC1	RC2	DI	RC1	RC2	DI	RC1	RC2	DI	RC1	RC2	DI	RC1	RC2
H7N9 Amino Acid Mutations/ Non-Synonymous Mutations																					
PB2	G293A	W98*		31.18			68.38			10.70			11.26			11.88			19.28		
PB2	A1172C	E391A																9.13			
PB2	G1699A	D567N										5.74									
PB2	A1757G	K586R																	11.04		
PB2	A2132G	N711S														14.75	99.54	99.33			
PB2	G2141A	S714N													13.00	99.48	98.39				
PB1	C413T	P138L												91.09	99.53						
PB1	G632A	R211K				10.16															
PB1	G769A	A257T				7.51															
PB1	T1309C	W437R																12.76			
PB1	G2197A	G733R							5.71												
PA	C812A	P271H																		13.33	
PA	G1027A	A343T				8.13														84.84	
PA	G1039A	D347N				9.65														83.92	
PA	A1046G	E349G																		79.83	
PA	A1172G	K391R								7.26											
PA	A1226G	N409S		7.52			6.50			7.53			10.28	99.80	100.00	12.01			7.85		
PA	G1342A	A448T					5.52														
PA	G1660A	V554I					6.90														
PA	T1718C	I573T					6.16														
PA	C1858T	P620S		9.27						7.94											
PA	G1867A	E623K		10.26						7.64						10.15			9.25		
PA	A2063G	E688G																	5.86		
HA	G199A	V67I (59)					7.55														
HA	A421G	N141D (133)	47.37	43.27			51.98			54.53			42.16	98.38	99.41	49.78	99.80	99.79	47.40	5.10	
HA	G427A	A143T (135)	12.55	11.06			8.78			6.46			7.50			8.46			13.70		
HA	G452A	G151E (144)		9.79			11.14			5.96			10.53			8.13			5.95	68.03	
HA	G499A	D167N (158)	31.59	36.72			41.31			28.73			38.22			28.18			30.34	94.82	
HA	G641A	G214E (205)	6.46				1.04														
HA	C683A	A228E (219)																		25.94	
HA	T704A*	L235Q* (226)		21.10			32.13			25.97			26.68	99.11	98.89	23.73	99.55	98.64	9.91		
HA	T1544C	V515A (505)	6.56	10.66			9.79			11.14			10.79			6.11			5.95		
NP	G350A	R117K					5.22														
NP	G850A	A284T	7.14										8.52			5.49					
NP	G1117A	A373T	13.99	20.20			11.25			23.57			17.84			20.01			14.32		
NP	C1289A	T430K																		5.11	
NA	C29T	T10I												99.17	99.55					13.93	
NA	C38T	T13I																			
NA	A844G	T282A														5.94					
NA	A1379G	K460R														99.30	100.00				
NA	A1195T	S399C								8.40											
MP	A977G	E326G					8.66														
NS	G133A	G45R	5.22																		
H7N9 Synonymous Mutations																					
PB2	C93T	I31	8.88				40.97					11.46				9.95			12.23	51.15	
PB2	C990T	F330	19.20	18.26			17.07					24.77				20.36			23.17		
PB2	A1077G	G359										6.01									
PB2	G1083A	E361	9.11	10.50			8.01					9.59				9.59			9.96		
PB2	C1134T	T378	5.35	8.26															6.06		
PB2	C1290T	P430	10.20				6.25					11.54				14.33			15.35		

PB2	G1329A	K443	6.02			5.75		9.35		9.39		8.51		10.62	
PB2	C1333T	L445				14.00								87.21	
PB2	C1359T	P453				7.87									
PB2	C1819T	L607	16.11	16.12				24.95		20.21		22.05		15.75	
PB2	C2142T	S714		10.30											
PB1	T183C	T61										7.86			
PB1	C297T	H99											99.22	99.76	
PB1	T693A	A231										11.02			
PB1	A750G	G250												8.52	
PB1	T1944C	A648				8.82									
PB1	G1968A	E656												82.73	
PA	C39T	I13						6.22							
PA	C156T	H52	8.29	4.74		19.90		4.79		7.17		10.30		19.78	
PA	G216A	L72	5.07	8.38								9.28		12.90	
PA	C325T	L109						5.22							
PA	G879A	E293									99.26	99.28			
PA	C888T	S296	5.20	7.96				7.54						8.22	
PA	C1005T	L335								6.30					
PA	C1497T	N499								6.37					
PA	G1812A	E604									95.13	97.20			
PA	A1830G	E610						13.22							
PA	T1875C	P625										5.23			
PA	G1884A	V628		5.99						7.04				23.35	8.33
PA	T2022C	D674	5.59			15.12		6.54						5.90	18.85
HA	C96T	T32 (24)	7.76	5.54		7.56								5.01	5.14
HA	C1197T	N399 (389)		5.26		8.85				9.59				6.60	
HA	G1326A	E396 (386)									79.05	87.35			
HA	G1401A	E467 (457)				8.63				10.29					
HA	C1554T	S518 (508)	9.79	7.52		8.35				5.84				7.06	12.89
NP	G885A	G295												7.59	
NP	C912T	F304													
NP	G1071A	Q357	13.88	22.09				25.79		19.32				20.66	16.28
NP	C1072T	L358												5.41	
NP	C1125T	D375													
NP	C1252T	N417	8.40			6.67		11.00							5.05
NA	G117A	P39						6.48							
NA	C825T	C275		9.41				7.49		5.80		8.65	99.76	99.52	10.70
NA	C1065T	N355								5.85					
MP	A297G	L99				6.14									
MP	C762T	A254				5.22									
NS	C154T	L52	17.36	18.56		14.87		16.42		17.79		17.16		17.07	84.72

Notes:

Displayed are mutations detected at a frequency $\geq 5\%$

HA number is displayed relative to start codon of H7, H3 numbering is indicated in parentheses

Red text denotes mutations that became enriched in multiple trios of ferrets

*Mutation was present in inoculum at less than 5% (2.26%)