

Table S5. List of All Non-Synonymous and Synonymous Mutations Identified During Sequential Transmission Experiments with A/harbor seal/New Hampshire/179629/2011 (H3N8)

Gene Segment	Nucleotide Change	Amino Acid Change Relative to Inoculum (for HA, (H3 numbering))	Nucleotide Frequencing																				
			Virus Inoculum	Trio 1: DI 8701->no transmission to RC1			Trio 2: DI A8704-> RC1 A8705-> RC2 A8706			Trio 3: DI A8707-> RC1 A8708-> RC2 A8709			Trio 4: DI A8712-> RC1 A8713-> RC2 A8714			Trio 5: DI 8251-> RC1 8252-> no transmission to RC2			Trio 6: DI A7717-> RC1 8258-> RC2 8285				
				DI	RC1	RC2	DI	RC1	RC2	DI	RC1	RC2	DI	RC1	RC2	DI	RC1	RC2	DI	RC1	RC2		
H3N8 Amino Acid Mutations/ Non-Synonymous Mutations																							
PB2	G2218A	D740N	9.76	12.61			9.91			5.86			8.24						9.07			8.08	9.38
PB1	A1130G	D377G																					
PB1	T2028G	N676K		17.28			13.30			10.07				25.54				90.46	16.45		11.55		
PB1	C2030G	T677R																7.41					
PA	G253A	A85T									7.82												
PA	T923C	I308T	5.11				9.26							5.85							6.57		
PA	G1184A	S395N																	99.05				
HA	G175A	V59I (43)								5.79													
HA	G433A	G145R (129)																100.00	99.55				
HA	G460A	A154T (138)						98.64	77.29													53.47	
HA	G460T	A154S (138)	10.43	33.93			46.34			44.63				58.02					30.18		28.84		
HA	A563G	D188G (172)									9.59												
HA	G668A	R223K (207)	29.48	14.73			13.04			9.43				3.69	97.66	98.16		11.38	98.97		10.50		
HA	A725G	Q242R (226)																					99.39
HA	A778G	M260V (244)						21.60															
NP	C1202T	A401V																					11.02
NA	C352T	P118S	37.53	37.40			31.98	82.73		35.13	100.00	98.61		46.61					33.95		28.68	86.22	98.92
NA	T958C	S320P	18.96	16.74			23.94			22.10				19.59					21.56		20.04		
NA	A1160T	Q387L								5.13													
NA	G1180A	V394I	35.14	40.82			47.38	15.71	93.81	40.00				42.41	100.00	99.77		32.91	99.77		46.54	9.68	
H3N8 Synonymous Mutations																							
PB2	A90C	I30								6.73									6.33				
PB2	G99A	K33								92.18													
PB2	C1044T	N348						79.20		96.94													
PB2	G1797A	L599																					
PB2	A2193G	V731	5.32	5.12			6.35			5.41				6.66								5.71	
PB1	G255A	T85						81.71															
PB1	G414A	P138																					
PB1	C813T	L271						78.38	6.23														
PB1	C1117T	L373																					
PB1	T1800C	N600																					
PA	A132G	V44	9.47	12.19			7.23	6.94		11.51				5.05					8.09		8.63		
PA	A147G	S49												9.45									
PA	A660G	P220						14.54	95.65														
PA	G1851A	E617					6.63																
HA	A432G	T144 (128)								7.80													
NP	G531A	G177																		100.00			
NP	A702G	A234											5.96										
NS	T240C	T80																					15.71

Notes:

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

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

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

PB2: At position 27 reference has G and Inoculum has A. No aa change.