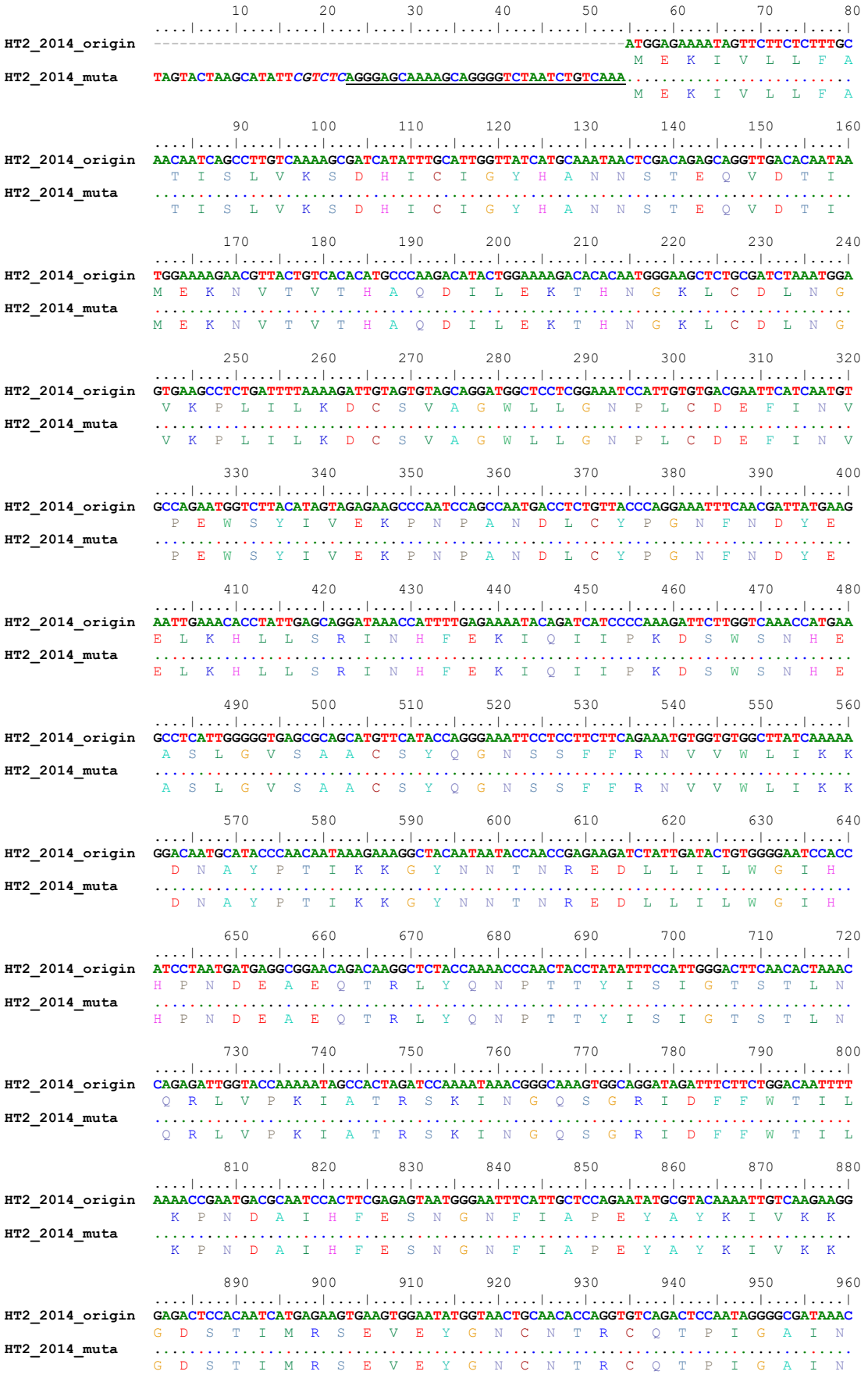
vaccines

MDPI

Supplementary Material



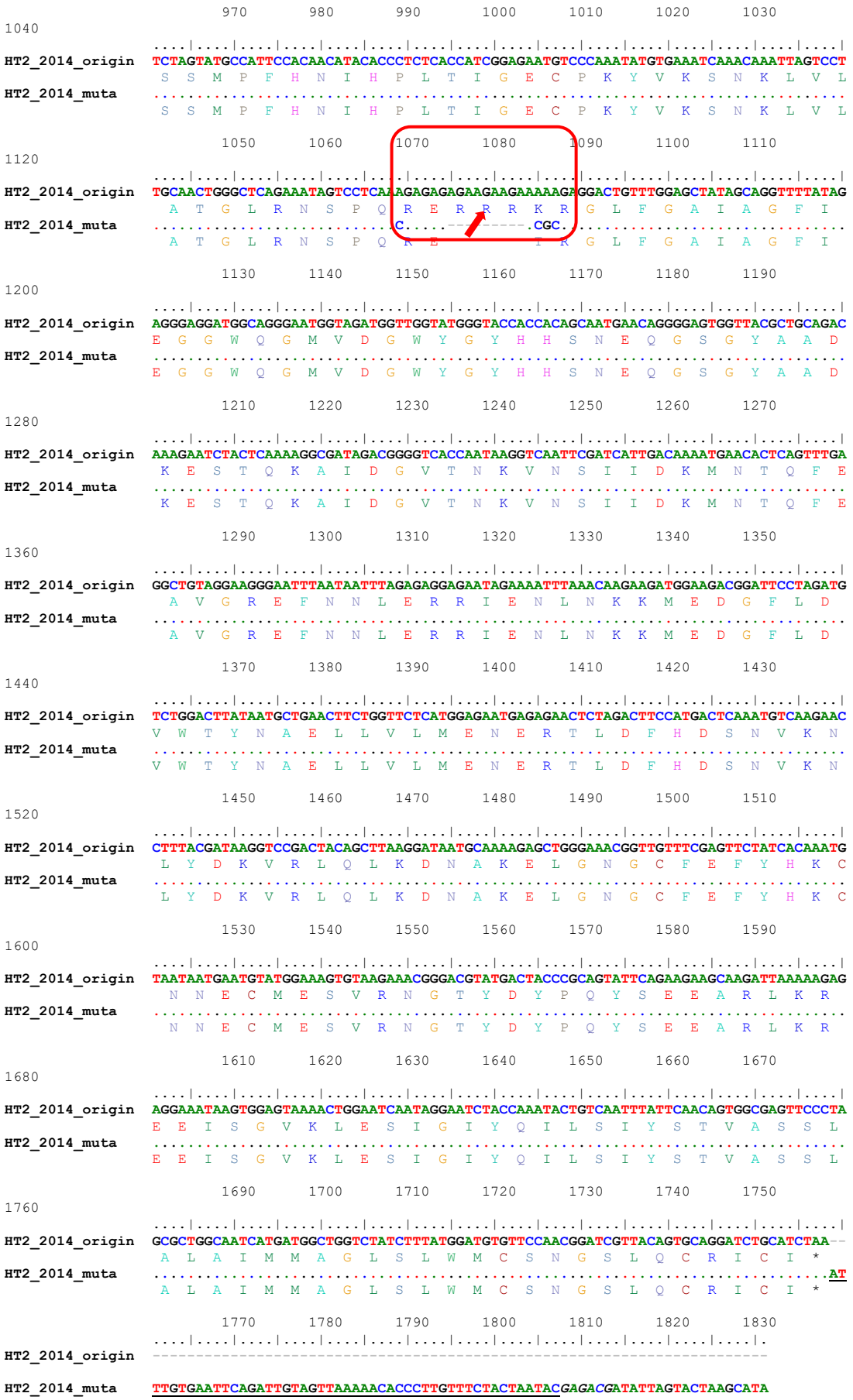

970 980 990 1000 1010 1020 1030
1040
HT2_2014_origin TCTAGTATGCCATTCCACAACATACACCCTCTCACCATCGGAGAATGTCCCAAATATGTGAAATCAAACAAATTAGTCCT
S S M P F H N I H P L T I G E C P K Y V K S N K L V L
HT2_2014_muta
S S M P F H N I H P L T I G E C P K Y V K S N K L V L
1050 1060 1070 1080 1090 1100 1110
1120
HT2_2014_origin TGCAACTGGGCTCAGAAATAGTCCTCAAAGAGAGAGAAGAAGAAAAAGAGGACTGTTTGGAGCTATAGCAGGTTTTATAG
A T G L R N S P Q R E R R R K R G L F G A I A G F I
HT2_2014_muta .....C.....CGC.....
A T G L R N S P Q R E T R G L F G A I A G F I
1130 1140 1150 1160 1170 1180 1190
1200
HT2_2014_origin AGGGAGGATGGCAGGGAATGGTAGATGGTTGGTATGGGTACCACCACAGCAATGAACAGGGGAGTGGTTACGCTGCAGAC
E G G W Q G M V D G W Y G Y H H S N E Q G S G Y A A D
HT2_2014_muta
E G G W Q G M V D G W Y G Y H H S N E Q G S G Y A A D
1210 1220 1230 1240 1250 1260 1270
1280
HT2_2014_origin AAAGAATCTACTCAAAAGGCGATAGACGGGGTCACCAATAAGGTCAATTCGATCATTGACAAAATGAACACTCAGTTTGA
K E S T Q K A I D G V T N K V N S I I D K M N T Q F E
HT2_2014_muta
K E S T Q K A I D G V T N K V N S I I D K M N T Q F E
1290 1300 1310 1320 1330 1340 1350
1360
HT2_2014_origin GGCTGTAGGAAGGGAATTTAATAATTTAGAGAGGAGAATAGAAAATTTAAACAAGAAGATGGAAGACGGATTCCTAGATG
A V G R E F N N L E R R I E N L N K K M E D G F L D
HT2_2014_muta
A V G R E F N N L E R R I E N L N K K M E D G F L D
1370 1380 1390 1400 1410 1420 1430
1440
HT2_2014_origin TCTGGACTTATAATGCTGAACTTCTGGTTCTCATGGAGAATGAGAGAACTCTAGACTTCCATGACTCAAATGTCAAGAAC
V W T Y N A E L L V L M E N E R T L D F H D S N V K N
HT2_2014_muta
V W T Y N A E L L V L M E N E R T L D F H D S N V K N
1450 1460 1470 1480 1490 1500 1510
1520
HT2_2014_origin CTTTACGATAAGGTCCGACTACAGCTTAAGGATAATGCAAAAGAGCTGGGAAACGGTTGTTTCGAGTTCTATCACAAATG
L Y D K V R L Q L K D N A K E L G N G C F E F Y H K C
HT2_2014_muta
L Y D K V R L Q L K D N A K E L G N G C F E F Y H K C
1530 1540 1550 1560 1570 1580 1590
1600
HT2_2014_origin TAATAATGAATGTATGGAAAGTGTAAGAAACGGGACGTATGACTACCCGCAGTATTCAGAAGAAGCAAGATTAAAAAGAG
N N E C M E S V R N G T Y D Y P Q Y S E E A R L K R
HT2_2014_muta
N N E C M E S V R N G T Y D Y P Q Y S E E A R L K R
1610 1620 1630 1640 1650 1660 1670
1680
HT2_2014_origin AGGAAATAAGTGGAGTAAAACTGGAATCAATAGGAATCTACCAAATACTGTCAATTTATTCAACAGTGGCGAGTTCCCTA
E E I S G V K L E S I G I Y Q I L S I Y S T V A S S L
HT2_2014_muta
E E I S G V K L E S I G I Y Q I L S I Y S T V A S S L
1690 1700 1710 1720 1730 1740 1750
1760
HT2_2014_origin GCGCTGGCAATCATGATGGCTGGTCTATCTTTATGGATGTGTTCCAACGGATCGTTACAGTGCAGGATCTGCATCTAA--
A L A I M M A G L S L W M C S N G S L Q C R I C I *
HT2_2014_muta .....AT
A L A I M M A G L S L W M C S N G S L Q C R I C I *
1770 1780 1790 1800 1810 1820 1830
HT2_2014_origin
HT2_2014_muta TTGTGAATTCAGATTGTAGTTAAAAACACCCTTGTTTCTACTAATACGAGACGATATTAGTACTAAGCATA

Figure 1. Stability of the artificial HA gene from the rescued virus was validated by sequencing. The 5′ and 3′ noncoding sequence in HT2_2014_muta were designed according to the molecular method described in Reference [14], including conserved regions between different influenza A subtypes (underlined) and the *BsmBI* recognition site (italic). The pathogenic region of HA from the original HPAI strain (HT2_2014_origin, IBT data collection) was modified as expected (HT2_2014_muta). The red arrow indicates the removal of the pathogenic region.

Table 1. Safety analysis of the rescued recombinant H5N1 virus in immunized chickens.

Immunization[a]	Total chicken number	Survival number	Chicken number with clinical outcomes[b]	Overall IVPI[c]
Control (PBS)	10	10/10	0/10	0
IBT-RG02	10	10/10	0/10	0
NAVET-Vifluvac[d]	10	10/10	0/10	0

[a]Vaccines were given to 3-week-old chickens via the intramuscular (IM) route as a single dose; [b]Clinical outcomes: chickens with symptoms of flu disease after immunization (according to body weight loss and OIE manual on assessment of sick and severely sick chickens); [c]The intravenous pathogenicity index (IVPI) was scored for each chicken and the mean value was calculated; [d]The NAVET-Vifluvac vaccine was used as positive control.