**S2 Table**. Sequences of optimized cDNA of wild type *Asfv*PolX and the primers for mutant *Asfv*PolX constructions

|  |  |
| --- | --- |
| **The optimized cDNA sequence of wild type *Asfv*PolXa** (from 5’ to 3’) | |
| *GGATCC*GGTGGTGGTATGCTGACCCTCATCCAGGGTAAAAAGATCGTTAACCACCTGCGTTCTCGTCTGGCGTTCGAATACAACGGTCAGCTCATCAAAATCCTGTCTAAAAACATCGTTGCGGTTGGTTCTCTGCGTCGTGAAGAAAAAATGCTGAACGACGTTGACCTGCTGATTATCGTACCAGAGAAGAAACTGCTCAAACACGTTCTGCCGAACATCCGTATTAAAGGTCTGTCTTTCTCTGTTAAAGTTTGTGGCGAGCGTAAATGCGTACTGTTCATCGAATGGGAAAAGAAAACCTACCAGCTCGACCTGTTCACCGCGCTGGCGGAAGAGAAACCGTACGCGATCTTCCATTTCACCGGTCCGGTGTCTTACCTGATCCGCATCCGTGCCGCTCTCAAAAAGAAGAACTACAAACTGAACCAGTACGGTCTGTTCAAAAACCAGACCCTGGTTCCGCTGAAAATCACTACTGAAAAGGAGCTGATCAAGGAACTCGGCTTCACCTACCGCATTCCGAAAAAACGTCTGTAA*CTCGAG* | |
| **Primers used for *Asfv*PolX mutant constructionsb** | |
| Name | Sequence (from 5’ to 3’) |
| PolX\_F | AAAGGATCCGGTGGTGGTATAGCTGACCCTCATCC |
| PolX\_R | AAACTCGAGTTACAGACGTTTTTTTCGGAATGCGG |
| PolX\_L52M\_F | GCTGAACGACGTTGACATGCTGATTATCGTACC |
| PolX\_L52M\_R | GGTACGATAATCAGCATGTCAACGTCGTTCAGC |
| PolX\_H115F\_F | GTACGCGATCTTCTTTTTCACCGGTCCGG |
| PolX\_H115F\_R | CCGGACCGGTGAAAAAGAAGATCGCGTAC |
| PolX\_H115D\_F | CCGTACGCGATCTTCGACTTCACCGGTCCGGTG |
| PolX\_H115D\_R | CACCGGACCGGTGAAGTCGAAGATCGCGTACGG |
| PolX\_H115E\_F | CCGTACGCGATCTTCGAGTTCACCGGTCCGGTG |
| PolX\_H115E\_R | CACCGGACCGGTGAACTCGAAGATCGCGTACGG |
| PolX\_V120A\_F | CACCGGTCCGGCGTCTTACCTGATCCGCATC |
| PolX\_V120A\_R | GATGCGGATCAGGTAAGACGCCGGACCGGTG |
| PolX\_L123A\_F | CACCGGTCCGGTGTCTTACGCGATCCGCATC |
| PolX\_L123A\_R | GATGCGGATCGCGTAAGACACCGGACCGGTG |
| PolX\_R125A\_F | CCGGTGTCTTACCTCGATCGCCATCCGTGCCGCTCTCAA |
| PolX\_R125A\_R | TTTGAGAGCGGCACGGATGGCGATCAGGTAAGACACCGG |
| PolX\_R127A\_F | CCTGATCCGCATCGCTGCCGCTCTCAAAAAG-5’ |
| PolX\_R127A\_R | CTTTTTGAGAGCGGCAGCGATGCGGATCAGG |
| PolX\_L163M\_F | GGAGCTGATCAAGGAAATGGGCTTCACCTACCGC |
| PolX\_L163M\_R | GCGGTAGGTGAAGCCCATTTCCTTGATCAGCTCC |
| PolX\_R168A\_R | AAACTCGAGTTACAGACGTTTTTTCGGAATGGCGTAGGTGAAGCCCAT |

**a**: *GGATCC* and *CTCGAG* at the 5'-end and 3'-end are BamHI and XhoI recognition sequence.*GGTGGTGGT* highlighted with underline codes for three Gly residues, it was designed to ensure the ULP1 cleavage efficiency.

**b**: PolX\_L52M\_F, PolX\_L52M\_R, PolX\_L163M\_F and PolX\_L163M\_R were used for the L52/163M mutant, via the site direct mutagenesis method. PolX\_F and PolX\_R168A\_R were used for the R168A mutant via regular PCR reaction. PolX\_F and PolX\_R were used as the two most outside primers for the overlap PCR reactions. which was utilized to construct all other mutants.