**S2 Table. Primers used to amplify regions within VACV and HPXV that flank *Bsa*I restriction endonuclease sites found in VACV (NC\_006998).**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Synthetic HPXV fragment** | **Primer Name** | **Primer sequence (5’ to 3’)** | **Position of *Bsa*I site in VACV [NC\_006998]** | **Position of *Bsa*I site in HPXV [DQ792504]** |
| Frag\_1A | HPXV 1A - FWD | CTGTATACCCATACTGAATTGATGAAC  | 16756 | 27,849 |
| HPXV 1A - REV | GAGTTAATATAGACGACTTTACTAAAGTCATG  |
| Frag\_1B | HPXV 1B - FWD | GGTTCTTTTTATTCTTTTAAACAGATCAATGG  | 23,076 | N/A |
| HPXV 1B - REV | TTCTTATTAAGACATTGAGCCCAGC  |
| Frag\_2 | HPXV 2A - FWD | AGTCATCAATCATCATTTTTTCACC  | 30,073 | 41,225 |
| HPXV 2A - REV | ATATAACGGACATTTCACCACC  |
| HPXV 2B – FWD | GTAACATATACAACTTTTATTATGGCGTC  | 45,485 | 56,778 |
| HPXV 2B – REV | CTAATCCACAAAAAATAGAATGTTTAGTTATTTTG |
| HPXV 2C – FWD | AGTGACTGTATCCTCAAACATCC  | 56,576 | 67,839 |
| HPXV 2C – REV | TTTATAAAGGGTTAACCTTTGTCACATC |
| Frag\_3 | HPXV 3A – FWD | TTGTGTAGCGCTTCTTTTTAGTC  | 60,981 | N/A |
| HPXV 3A – REV | AAACGGATCCATGGTAGAATATG  |
| HPXV 3B – FWD | TATTTGCATCTGCTGATAATCATCC  | 84,916 | 84,353 |
| HPXV 3B – REV | CGATGGATTCAAATGACTTGTTAATG  |
| Frag\_4 | HPXV 4A – FWD | ATGCCTTTACAGTGGATAAAGTTAAAC  | 85,101 | 96,243 & 96,428 |
| HPXV 4A – REV | CTGGATCCTTAGAGTCTGGAAG  |
| HPXV 4B – FWD | CGGAAAATGAAAAGGTACTAGATACG  | 98,134 | 109,485 |
| HPXV 4B – REV | TGAATAGCCGTTAAATAATCTATTTCGTC  |
| HPXV 4C – FWD | TATGGATACATTGATAGCTATGAAACG  | 99,302 & 99,481 | 110,653 & 110,832 |
| HPXV 4C – REV | AATACATCTGTTAAAATTGTTTGACCCG  |
| Frag\_5 | HPXV 5A – FWD | CATTTTATTTCTAGACGTTGCCAG  | 111,686 | 123,037 |
| HPXV 5A – REV | CGATATGAAACTTCAGGCGG  |
| HPXV 5B – FWD | ACAAAACGATTTAATTACAGAGTTTTCAG  | 122,484 | N/A |
| HPXV 5B – REV | GTCCGGTATGAGACGACAG  |
| HPXV 5C – FWD | TTAGGGATCACATGAATGAAATTCG  | 133,505 | 144,838 |
| HPXV 5C – REV | TATGGAAGTTCCGTTTCATCCG  |
| HPXV 5D – FWD | GACTTGATAATCATATATTAAACACATTGGATC  | 138,306 | 149,718 |
| HPXV 5D – REV | AGATCTCCAGATTTCATAATATGATCAC  |
| Frag\_6 | HPXV 6A – FWD | ATGATACGTACAATGATAATGATACAGTAC  | 163,521 | 175,062 |
| HPXV 6A – REV | TGATTTTTGCAATTGTCAGTTAACACAAG  |
| Frag\_7 | HPXV 7A – FWD | TACTGTACCCACTATGAATAACGC  | 169,035 | 180,578 |
| HPXV 7A – REV | GATATCAACATCCACTGAAGAAGAC  |
| HPXV 7B – FWD | ATCTTACCATGTCCTCAAATAAATACG  | 175,849 | 187,467 |
| HPXV 7B – REV | ATAGCTCTAGGTATAGTCTGCAAG  |
| HPXV 7C – FWD | GCGAACTCCATTACACAAATATTTG  | 181,952 | 195,683 |
| HPXV 7D - REV | GATGTTTCTAAATATAGGTTCCGTAAGC  |