

Plasmid	Construct Info	Forward Primer	Reverse Primer
pJB02	HSV1 UL34 1-220	5'-aaaaaag <u>tcgac</u> cctatggcgggactgggcaag-3' Sall	5'-aaaaaagcggccgccttcagccccctggccc-3' NotI
pJB57	HSV1 UL34 1-220-His <sub>8</sub>	5'-aaaaaag <u>tcgac</u> cctatggcgggactgggcaag-3' Sall	5'-aaaaaagcggccgccttcagccccctggccc-3' NotI
pJB84	HSV UL34 1-185-His <sub>8</sub>	5'-aaaaaag <u>tcgac</u> cctatggcgggactgggcaag-3' Sall	5'-aaaaaagcggccgccttcagccccctggccc-3' NotI
pKH90	HSV1 UL31 f.l.	5'-agcaggatcctatgacaccgacccccat-3' BamHI	5'-aaatcgccgccttacggcggaggaaactc-3' NotI
pKH86	HSV1 UL31 51-306	5'-agcaggatcccaggagctgtgtttacac-3' BamHI	5'-aaatcgccgccttacggcggaggaaactc-3' NotI
pJB41	HSV1 UL31 41-306	5'-aaaaaag <u>gatccc</u> ggaagagcctgccg-3' BamHI	5'-aaatcgccgccttacggcggaggaaactc-3' NotI
pMT02	HSV1 UL31-Δ40 R49S/K50S	5'- <b>agc</b> caggagctgtgtttacacgagcgc-3'	5'- <b>gct</b> ggcgtgaggcggcaggctctt-3'
pMT08	HSV1 UL31-Δ40 R41S/K42S/S43R/L44K/R49S/K50S	5'- <b>cgtaa</b> accgctcacgccagc-3'	5'- <b>gctgct</b> ggaggatccgggccc-3'
pMT09	HSV1 UL31-Δ40 R41S/K42S/P45R/P46K/R49S/K50S	5'- <b>gctgct</b> ggaggatccgggccc-3'	5'-agcct <b>gctaa</b> cacgccagcag-3'
pMT10	HSV1 UL31-Δ40 R41S/K42S/H47R/A48K/R49S/K50S	5'-agcctgccgcct <b>cgtaa</b> agc-3'	5'- <b>gctgct</b> ggaggatccgggccc-3'
pMT03	HSV1 UL31-Δ40 R41S/K42S	5'-aaaaaag <u>gatccc</u> tctctctagcctgccgc-3' BamHI	5'-aaatcgccgccttacggcggaggaaactc-3' NotI
pMT01	HSV1 UL31-Δ40 R41S/K42S/R49S/K50S	5'-cctcacgcct <b>ctctc</b> caggagctgtgtt-3'	5'-cggcaggct <b>agaag</b> aggaggatc-3'
pMT17	HSV1 UL31-Δ40 L44A	5'-agc <b>gcg</b> ccgcctcacgcc-3'	5'-ctccgggatccgggccc-3'
pMT11	HSV1 UL31-Δ40 P45A/P46A	5'-agcct <b>ggcggc</b> gcacgccccaaa-3'	5'-ctccgggatccgggccc-3'
pMT18	HSV1 UL31-Δ50 RKRK	5'-agctgtgtctgcacgagcgc-3'	5'-cctgttt <b>gcttac</b> gggatccggg-3'
pMT35	HSV1 UL31-Δ40 Scr 41-50	5'- <b>aaaagccc</b> aaactgcatctg <b>cgcg</b> tc <b>ccg</b> caggag ctgtttacacgagcgc-3'	5'-ggatccgggcccctggaaca-3'
pMT04	HSV1 UL31-Δ40 S43E	5'- <b>gaact</b> gccgcctcacgc-3'	5'-ctccgggatccgggccc-3'
pJB60	HSV1 UL31 2-306 S11E/S24E/S26E/S27E/S40E/S43E	5'- ccccatcgccgcggc <b>gaa</b> cggcccggccctacac- 3'	5'- gtgatagggcccgccg <b>ttc</b> gccgcggcgatgggg- 3';
	Round 2	5'- gagcgcggcgg <b>gaa</b> cgc <b>gaaga</b> agcggccggcggg- 3'	5'-cccgcggccg <b>cttctc</b> g <b>gtt</b> ccgccggcgctc- 3'
	Round 3	5'- cgtggtgctcggg <b>ccgaa</b> cggaa <b>gaa</b> ctgccgcctc acgcc-3'	5'- gggcgtagggcggcag <b>ttc</b> ctc <b>gtt</b> cgcccgacgc accacg-3'
pMT16	HSV-1 UL31 S11E/S24E/S43E	5'-gcaaggagcgcggcgg <b>gaa</b> cgc-3'	5'-cgtgataggcccgccg <b>ttc</b> gcc-3'
	Round 2	5'-gggccctatcacgcaaggagc-3'	5'-gggcc <b>gtt</b> cggcggcgatg-3'
pMT19	HSV-1 UL31 1-50	5'-aaaaaag <u>gatccc</u> tctctctatgacaccgacccc-3' BamHI	5'- aaaaaagcggccgcctattatttgcggcgtgaggcg-3' NotI
pMT29	HSV-1 UL31 C1-50	5'-ctgtccaggggcccgatc <b>tg</b> ctatg-3'	5'-catag <b>cagg</b> atccgggcccctggaacag-3'
	Round 2	5'-aggggcccggatc <b>tg</b> ctatgacaccgacc-3'	5'-ggtcgtgtcatag <b>cagg</b> atccgggcccct-3'
	Round 3	5'-cagagattcaatgctgaaggagcgt-3'	5'-accgctctcaagcattgaaatctctg-3'
pMT33	HSV-1 UL31 1-C51	5'-cacgccgcaaat <b>gcta</b> ataagcgc-3'	5'-gccgctattag <b>catt</b> gcggcg-3'

**Supplementary Table S3. List of primers used for cloning described in Materials and Methods.** All primers are listed in the 5'-3' direction. Mutations are bolded, restriction digest sites are underlined and listed underneath applicable primers.