# Supplemental Material: Section 1: Detailed ORF Coordinates Data for Individual EEHV PCR Loci:

## Table S1: Details of Sequenced EEHV3, EEHV4, EEHV5A, EEHV5B and EEHV6 Gene Coding Regions:

1. EEHV3 [#NAP27, Hansa]: Five segments, POL, TERex3, EXO-gM, OBP & POR-HEL

Total = 4,117-bp Differences = 1,509-bp = 37%

| Virus No. Gene/ORF | HCMV ORF | HSV ORF | Ori- ent | Protein Name | Position No | Gene Size | Protein Size | Genbank Acc # | Status | Kimba Cordinates | Diff % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| U38 | UL54 | UL30 | F | POL | 00001-01253 | (1253) | (419) | JN983092 | Core | 077777-079002 | 34 |
| U60ex3 | UL89ex2 | UL15ex2 | R | TERex3 | 00316-00001 | (316) | (106) | EU658937* | Core | 123721-124036 | 22 |
| U70 | UL98 | UL12 | F | EXO | 00001-00041 | (41) | (13) | JN983093 | Core | 132981-133575 | 51 |
| U71 | UL99 | UL11 | F | myrTeg | 00001-00295 | (295) | (98) | | Core | | |
| U72 | UL100 | UL10 | R | gM | 00746-00460 | (284) | (95) | | Core | | |
| U73/ORF-G | Nil | UL09 | F | OBP | 00001-00892 | (892) | (297) | JN983094 | α/β2 | 134651-135422 | 42 |
| U76 | UL104 | UL06 | R | POR | 00328-00001 | (328) | (109) | JN983095 | Core | 140968-141878 | 30 |
| U77 | UL105 | UL05 | F | HEL | 00279-00911 | (632) | (211) | | | | |

2. EEHV4 [#NAP22]: Four segments, POL, TERex3, EXO-gM-OBP & POR-HEL.

Total = 5,743-bp Differences = 2,574-bp = 48%

| Virus No. Gene/ORF | HCMV ORF | HSV ORF | Ori- ent | Protein Name | Position No | Gene Size | Protein Size | Genbank Acc # | Status | Kimba Cordinates | Diff % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| U38 | UL54 | UL30 | F | POL | 00001-01236 | (1236) | (412) | JN983096 | Core | 077782-079008 | 34 |
| U60ex3 | UL89ex2 | UL15ex2 | R | TERex3 | 00316-00001 | (316) | (106) | EU658935* | Core | 123721-124036 | 23 |
| U70 | UL98 | UL12 | F | EXO | 00001-00041 | (41) | (13) | JN983097 | Core | 132981-135284 | 46 |
| U71 | UL99 | UL11 | F | myrTeg | 00001-00289 | (289) | (96) | | Core | | |
| U72 | UL100 | UL10 | R | gM | 01603-00485 | 1118 | 372 | | Core | | |
| U73/ORF-G | Nil | UL09 | F | OBP | 02109-03314 | (1205) | (401) | | A/B2 | | |
| U76 | UL104 | UL06 | R | POR | 00311-00001 | (311) | (104) | JN983099 | Core | 140984-141861 | 32 |
| U77 | UL105 | UL05 | F | HEL | 00262-00877 | (616) | (205) | | Core | | |

3. EEHV5A[#NAP28]: Nine segments, gB-POL, U33, RRA-RRB, gH-TK, vGPCR, MCP, EXO-gM, OBP, & POR-HEL.

Total = 14,615-bp Differences = 3,750-bp = 25.7%

| Virus No. Gene/ORF | HCMV ORF | HSV ORF | Ori- ent | Protein Name | Position No | Gene Size | Protein Size | Genbank Acc # | Status | Kimba Cordinates | Diff % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| U39 | UL55 | UL29 | F | gB | 00001-02504 | (2504) | (835) | JN983100 | Core | 073998-079043 | 27 |
| U38 | UL54 | UL30 | F | POL | 02515-04975 | (2490) | (830) | | Core | | |
| U33 | UL49 | Nil | F | Cys-R | 00001-00530 | (530) | (177) | JN983101 | β/γ | 083622-084193 | 33 |
| U28 | UL45 | UL39 | F | RRA | 00001-01093 | (1093) | (331) | JN983102 | Core | 098665-100366 | 26 |
| U27.5/ORF-H | Nil | UL40 | F | RRB | 01106-01668 | (562) | (154) | | α/γ | | |
| U48 | UL75 | UL22 | R | gH | 00533-00001 | (534) | (145) | JN983103 | Core | 105379-106922 | 32 |
| U48.5/ORF-E | Nil | UL23 | R | TK | 01564-00530 | 1034 | 344 | | α/γ | | |
| U49 | UL76 | UL24 | F | | 01566-01578 | (12) | (4) | | Core | | |
| U51 | UL78 | Nil | F | vGPCR1 | 00001-00697 | (697) | (232) | KC854716 | β | 109412-110092 | 33 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| U57 | UL86 | UL19 | R | MCP | 02244-00001 | (2244) | (738) | JN983104 | Core | 115575-117818 | 24 |
| U70 | UL98 | UL12 | F | EXO | 00001-00032 | (32) | (9) | JN983105* | Core | 132987-133601 | 36 |
| U71 | UL99 | UL11 | F | myrTeg. | 00001-00283 | (283) | (93) | | Core | | |
| U72 | UL100 | UL10 | R | gM | 00657-00366 | (292) | (96) | | Core | | |
| U73/ORF-G | Nil | UL09 | F | OBP | 00001-00758 | (758) | (253) | JN983106 | α/β2 | 134631-135388 | 22 |
| U76 | UL104 | UL06 | R | POR | 01490-00001 | (1490) | (497) | JN983107 | Core | 139806-141313 | 21 |
| U77 | UL105 | UL05 | F | HEL | 01489-01508 | (20) | (7) | | Core | | |

## 4. EEHV5A[#NAP50]: Twelve segments, gB-POL, U33, RRA-PPF, gH-TK, gH to PAC2, vGPCR1, MCP, TERex3, EXO-gM, OBP, POR-HEL, HEL to ORF-O and ORF-K to ORF-L.

Total = 26,090-bp Differences = 8,294-bp = 31.8%

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| U39 | UL55 | UL29 | F | gB | 00001-02515 | (2515) | (838) | JN983108 | Core | 073982-079038 | 27 |
| U38 | UL54 | UL30 | F | POL | 02526-04981 | (2455) | (818) | | Core | | |
| U33 | UL49 | Nil | F | Cys-rich | 00001-00520 | (520) | (172) | JN983109 | β/γ | 083622-084183 | 34 |
| U28 | U45 | UL39 | F | RRA | 00001-01121 | (1121) | (374) | JN983110 | Core | 098636-101613 | 28 |
| U27.5/ORF-H | Nil | UL40 | F | RRB | 01134-02039 | 906 | 301 | | α/γ | | |
| U27/ORF-I | UL44 | UL42 | F | PPF | 02283-02941 | (658) | (219) | | Core | | |
| U48 | UL75 | UL22 | R | gH | 00516-00001 | (516) | (172) | JN983111 | Core | 105396-108178 | 29 |
| U48.5/ORF-E | Nil | UL23 | R | TK | 01547-00513 | 1034 | 344 | | α/γ | | |
| U49 | UL76 | UL24 | F | | 01549-02247 | 699 | 232 | | Core | | |
| U50 | UL77 | UL25 | F | PAC2 | 02066-02850 | (784) | (261) | | Core | | |
| U51 | UL78 | Nil | F | vGPCR1 | 00001-00875 | (875) | (292) | KC854743 | β | 109231-110088 | 33 |
| U57 | UL86 | UL19 | R | MCP | 01640-00001 | (1640) | (537) | JN983112 | Core | 116179-117818 | 25 |
| U60ex3 | UL89ex2 | UL15ex2 | R | TERex3 | 00507-00001 | (507) | (169) | KC854717 | Core | 123677-124434 | 24 |
| U62 | UL91 | Nil | F | | 00524-00747 | (224) | (78) | | β/γ | | |
| U70 | UL98 | UL12 | F | EXO | 00001-00055 | (55) | (18) | JN983113* | Core | 132963-133613 | 31 |
| U71 | UL99 | UL11 | F | myrTeg. | 00001-00300 | (300) | (100) | | Core | | |
| U72 | UL100 | UL10 | R | gM | 00686-00383 | (303) | (101) | | Core | | |
| U73/ORF-G | Nil | UL09 | F | OBP | 00001-00812 | (812) | (271) | JN983114 | α/β2 | 134614-135424 | 21 |
| U76 | UL104 | UL06 | R | POR | 01481-00001 | (1481) | (494) | JN983115 | Core | 139815-141793 | 22 |
| U77 | Ul105 | UL05 | F | HEL | 01480-01982 | (502) | (187) | | Core | | |
| U77 | UL105 | UL05 | F | HEL | 00001-00023 | (23) | (8) | KC854719 | Core | 143644-148727 | 35 |
| U77.5/ORF-M | Nil | Nil | F | Nuclear | 00356-01810 | 1455 | 484 | | Novel | | |
| U80.5/ORF-N | Nil | Nil | R | vCXCL1 | 02237-01917 | 321 | 106 | | Novel | | |
| U81 | UL114 | UL02 | R | UDG | 03256-02282 | 975 | 324 | | Core | | |
| U82 | UL115 | UL01 | R | gL | 04061-03222 | 840 | 279 | | Core | | |
| U82.5/ORF-Oex3 | Nil | Nil | R | S/TGlyP | 04536-03985 | (551) | (184) | | Novel | | |
| U82.5/ORF-Oex2 | Nil | Nil | R | S/TGlyP | 04825-04623 | (202) | (67) | | Novel | | |
| U82.5/ORF-Oex1 | Nil | Nil | R | S/TGlyP | 04984-04900 | (840) | (28) | | Novel | | |
| U85.5/ORF-Kex3 | Nil | Nil | R | SplGlyP | 02032-00001 | (2032) | (677) | KC854744 | Novel | 152042-155356 | 39 |
| U85.5/ORF-Kex2 | Nil | Nil | R | SplGlyP | 02158-02226 | (68) | (23) | | Novel | | |
| U85.5/ORF-Kex1 | Nil | Nil | R | SplGlyP | 02380-02413 | (34) | (11) | | Novel | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| U86.5/ORF-L | Nil | Nil | R | SplGlyP | 03137-02834 | (303) | (100) | | Novel | | |

### 5. EEHV5B[#NAP58TW] Ten segments: gB-POL, U33, RRA-PPF, gH to vGPCR1, MCP, U71-gM, OBP, TERex3, POR-HEL, HEL to ORF-L. Total = 29,347-bp Differences = 10,241-bp = 34.9%

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| U39 | UL55 | UL29 | F | gB | 00001-02500 | (2500) | (833) | JX011013 | Core | 073997-078863 | 27 |
| U38 | UL54 | UL30 | F | POL | 02511-04791 | (2280) | (760) | | Core | | |
| U33 | UL49 | Nil | F | Cys-rich | 00001-00599 | (599) | (200) | KC854748 | β/γ | 083521-084162 | 32 |
| U28 | UL45 | UL39 | F | RRA | 00001-01120 | (1120) | (373) | KC854749 | Core | 098637-101609 | 27 |
| U27.5/ORF-H | Nil | UL40 | F | RRB | 01133-02038 | 906 | 301 | | α/γ | | |
| U27/ORF-I | UL44 | UL42 | F | PPF | 02286-02932 | (648) | (216) | | Core | | |
| U48 | UL75 | UL22 | R | gH | 00518-00001 | (518) | (173) | KC854722 | Core | 105394-110158 | 31 |
| U48.5/ORF-E | Nil | UL23 | R | TK | 01549-00515 | 1034 | 345 | | A/G | | |
| U49 | UL76 | UL24 | F | - | 01551-02249 | 699 | 232 | | Core | | |
| U50 | UL77 | UL25 | F | PAC2 | 02068-03837 | 1770 | 589 | | Core | | |
| U51 | UL78 | Nil | F | vGPCR1 | 03876-04882 | (1007) | (338) | | B | | |
| U57 | UL86 | UL19 | R | MCP | 00001-01139 | (1139) | (380) | KC854751 | Core | 116671-117809 | 27 |
| U60ex3 | UL89ex2 | UL15ex2 | R | TERex3 | 00510-00001 | (510) | (170) | KC854752 | Core | 123674-124434 | 24 |
| U62 | UL91 | Nil | F | | 00527-00750 | (224) | (75) | | β/γ | | |
| U70 | UL98 | UL12 | F | EXO | 00001-00047 | (47) | (16) | JX011021* | Core | 132971-133612 | 32 |
| U71 | UL99 | UL11 | F | myrTeg. | 00001-00295 | (295) | (98) | | Core | | |
| U72 | UL100 | UL10 | R | gM | 00680-00378 | (303) | (101) | | Core | | |
| U73/ORF-G | Nil | UL09 | F | OBP | 00001-00826 | (826) | (275) | KC854753 | α/β2 | 134605-135430 | 21 |
| U76 | UL104 | UL06 | R | POR | 01538-00001 | (1538) | (513) | JX011029 | Core | 139758-141788 | 22 |
| U77 | Ul105 | UL05 | F | HEL | 01492-02034 | (542) | (181) | | Core | | |
| U77 | UL105 | UL05 | F | HEL | 00001-00028 | (28) | (8) | KC854723 | Core | 143638-155354 | 42 |
| U77.5/ORF-M | Nil | Nil | F | Nuclear | 00359-01807 | 1449 | 482 | | Novel | | |
| U80.5/ORF-N | Nil | Nil | R | vCXCL1 | 02186-01875 | 312 | 103 | | Novel | | |
| U81 | UL114 | UL02 | R | UDG | 03165-02224 | 942 | 313 | | Core | | |
| U82 | UL115 | UL01 | R | gL | 03973-03131 | 843 | 280 | | Core | | |
| U82.5/ORF-Oex3 | Nil | Nil | R | S/TGlyP | 04548-03891 | (658) | (218) | | Novel | | |
| U82.5/ORF-Oex2 | Nil | Nil | R | S/TGlyP | 04743-04617 | (127) | (42) | | Novel | | |
| U83.5/ORF-Oex1 | Nil | Nil | R | S/TGlyP | 05600-04821 | (780) | (260) | | Novel | | |
| U83.5/ORF-Pex2 | Nil | Nil | R | S/TGlyP | 07035-05696 | (1340) | (446) | | Novel | | |
| U83.5/ORF-Pex1 | Nil | Nil | R | S/TGlyP | 07348-07105 | (244) | (81) | | Novel | | |
| U84.5/ORF-Q | Nil | Nil | | (absent) | | | | | | | |
| U85.5/ORF-Kex3 | Nil | Nil | R | SplGlyP | 09565-07586 | (1979) | (659) | | Novel | | |
| U85.5/ORF-Kex2 | Nil | Nil | R | SplGlyP | 09688-09650 | (68) | (23) | | Novel | | |
| U85.5/ORF-Kex1 | Nil | Nil | R | SplGlyP | 09900-09814 | (87) | (29) | | Novel | | |
| U86.5/ORF-L | Nil | Nil | R | IE-like | 10784-10491 | (294) | (97) | | Novel | | |

### 6. EEHV6 [#NAP35]: Thirteen segments, gB-POL, U33, RRA-gO, gH-TK, gH-PAC2, vGPCR1, MCP, TERex3-U63, TERex1,2, EXO-gM, OBP, POR-HEL, HEL to ORF-O and ORF-K to ORF-L. Total = 31,828-bp Differences = 5,559-bp = 17.4%

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| U39 | UL55 | UL29 | F | gB | 00001-02541 | 2541 | 847 | JF692762 | Core | 073959-079043 | 17 |
| U38 | UL54 | UL30 | F | POL | 02563-05059 | (2496) | (832) | | Core | | |

|  |  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|
| U33 | UL49 | Nil | F | Cys-rich | 00001-00916 | (916) | (305) | JN983116 | β/γ | 083537-084442 | 21 |
| U28 | UL45 | UL39 | F | RRA | 00001-01131 | (1131) | (377) | JN983117 | Core | 098630-103703 | 17 |
| U27.5/ORF-H | Nil | UL40 | F | RRB | 01173-02078 | 906 | 302 |  | A/B2 |  |  |
| U27/ORF-I | UL44 | UL42 | F | PPF | 02319-03530 | 1212 | 403 |  | Core |  |  |
| U45.7/ORF-J | Nil | Nil | F |  | 03602-04100 | 498 | 165 |  | Novel |  |  |
| U46 | UL73 | UL49A | F | gN | 04084-04374 | 292 | 97 |  | Core |  |  |
| U47/ORF-D | UL74 | Nil | R | gO | 05024-04396 | (628) | (209) |  | β |  |  |
| U48 | UL75 | UL22 | R | gH | 00539-00001 | (539) | (179) | JN983119 | Core | 105364-108084 | 19 |
| U48.5/ORF-E | Nil | UL23 | R | TK | 01551-00472 | 1080 | 359 |  | α/γ |  |  |
| U49 | UL76 | UL24 | F |  | 01553-02251 | 699 | 232 |  | Core |  |  |
| U50 | UL77 | UL25 | F | PAC2 | 02082-02706 | (636) | (212) |  | Core |  |  |
| U51 | UL78 | Nil | F | vGPCR1 | 00001-00833 | (833) | (277) | JN983120 | β | 109404-110239 | 21 |
| U57 | UL86 | UL19 | R | MCP | 02244-00001 | (2244) | (748) | JN983121 | Core | 115577-117820 | 17 |
| U60ex3 | UL89 | UL15ex2 | R | TERex3 | 00583-00001 | (583) | (194) | JN983122 | Core | 123601-124519 | 13 |
| U62 | UL91 | Nil | F |  | 00607-00873 | 267 | 88 |  | β/γ |  |  |
| U63 | UL92 | Nil | F |  | 00821-00915 | (94) | (31) |  | β/γ |  |  |
| U66ex2 | Nil | Nil | R | TERex2 | 00082-00001 | (82) | (27) | JN983123 | Core | 127386-127713 | 14 |
| U66ex1 | UL89ex1 | UL15ex1 | R | TERex1 | 00164-00325 | (161) | (54) |  | Core |  |  |
| U70 | UL98 | UL12 | F | EXO | 00001-00052 | (52) | (17) | JN983124 | Core | 132966-133555 | 19 |
| U71 | UL99 | UL11 | F | myrTeg. | 00001-00291 | (291) | (97) |  | Core |  |  |
| U72 | UL100 | UL10 | R | gM | 00719-00362 | (357) | (118) |  | Core |  |  |
| U73/ORF-G | Nil | UL09 | F | OBP | 00001-00801 | (801) | (267) | JN983125 | α/β2 | 134615-135415 | 14 |
| U76 | UL104 | UL06 | R | POR | 01298-00001 | (1298) | (433) | JN983126 | Core | 139998-141864 | 13 |
| U77 | UL105 | UL05 | F | HEL | 01297-01827 | (531) | (176) |  | Core |  |  |
| U77 | UL105 | UL05 | F | HEL | 00001-00023 | (23) | (7) | KC854726 | Core | 143644-147936 | 19 |
| U77.5/ORF-M | Nil | Nil | F | Nuclear | 00329-01825 | 1497 | 498 |  | Novel |  |  |
| U80.5/ORF-N | Nil | Nil | R | vCXCL1 | 02252-01956 | 297 | 98 |  | Novel |  |  |
| U81 | UL114 | UL02 | R | UDG | 03312-02356 | 957 | 318 |  | Core |  |  |
| U82 | UL115 | UL01 | R | gL | 04183-03278 | 906 | 301 |  | Core |  |  |
| U82.5/ORF-Oex3 | Nil | Nil | R | S/TGlyP | 04261-04026 | (235) | (78) |  | Novel |  |  |
| U85.5/ORF-Kex3 | Nil | Nil | R | SplGlyP | 01974-00001 | (1974) | (656) | JX011085 | Novel | 152200-158615 | 20 |
| U85.5/ORF-Kex2 | Nil | Nil | R | SplGlyP | 02128-02061 | (68) | (23) |  | Novel |  |  |
| U85.5/ORF-Kex1 | Nil | Nil | R | SplGlyP | 00331-02355 | (25) | (8) |  | Novel |  |  |
| U86.5/ORF-L | Nil | Nil | R | IE-like | 06198-02684 | (3515) | (1171) |  | Novel |  |  |

Core = common to all herpesvirus sub-families; α/β = Alpha & Beta only; β/γ = Beta & Gamma only; β = Beta only; α/β2 = Alpha & *Roseoloviruses* only; Novel = not found in any other herpesviruses;
* = Unchanged from previous reports (3,17).
( ) = Incomplete ORF or exon only.
Diff % = level of nucleotide divergence for each locus compared to EEHV1A(Kimba).

## Supplemental Material: Section 2: Examples of Primers Used to Amplify Specific Gene Loci:

Three sets of two-step moderately-redundant PAN-Herpesvirus DNA PCR primers for small segments of either the POL or TER gene loci were used for initial detection of various EEHV genomes when present at high abundance in acute disease blood or necropsy tissue samples. Those primers (1), as well as the PAN EEHV and EEHV1, EEHV2, EEHV3, EEHV4, EEHV5 and EEHV6-specific diagnostic POL or TER locus primers have all been described elsewhere (3,4). Several of the POL, U71-U72(gM), U76(POR)-U77(HEL), and U51(vGPCR1) primer sets presented in Stanton et al (19) 2013 as well as the U47.5(TK)-U48(gH) primers used in Zachariah et al (6) were also effective for EEHV6. The same initial U38(POL) and U71-U72(gM) primer sets above were also effective for EEHV5 as reported in Alkins et al (17). Other selected specific or consensus primer sets that were designed for and found effective for amplification of key gene segments that are referred to in detail in the text are listed below. Detailed information about numerous other primers (not listed) that were used for primer PCR, cycle sequencing and phage lambda genome walking procedures can be obtained from the corresponding author.

### EEHV1,2,5,6 Common LHS-1 Extended U38(POL-N terminal) Locus:

| | | |
|---|---|---|
| R1 | LGH8582 | 5’-GGGTACACTGCACTGAGATCTATGAAC-3’ |
| R2 | LGH8583 | 5’-CGCGAGATAGGTCAGTGGCCCGTAAAGG-3’ |
| L2 | LGH8584 | 5’-CTTTTCCCACAAAGTGATTAGAGAAAC-3’ |
| L1 | LGH8585 | 5’-CACATGATATCTGAATGATTATATCGTCCG-3’ |

First round R1-L1 = 1.1-kb, second round A R1-L2 = 800-bp, second round B R2-L1 = 850-bp, third round AB = 500-bp.

### EEHV1,2,5,6 Common LHS-2 Extended U38(POL-N terminal) Locus:

| | | |
|---|---|---|
| R1 | LGH8450 | 5’-CCATATCTGATG[A/T]TGAGTTTCTCTAATCACTTTG-3’ |
| R2 | LGH8451 | 5’-CGAGTTCTGTTCGTTTGGATGGTATTCTC-3’ |

L2 LGH8452 5’-GAGCTCRTATTCYGA[G/T]GCACATTCGTATATG

L1 LGH8453 5’-GGATACCTTATCCATACACACGCGATCATATGTC-3’

First round R1-L1 = 700-bp, second round A R1-L1 = 600-bp, second round R2-L1 = 450-bp, third round AB = 280-bp

EEHV1,2,5,6 Common LHS-3 Extended U38(POL-N terminal) Locus:

R1 LGH8423 5’-CATATACGAATGTGCCTCRGAATAYGAG-3’

R2 LGH8424 5’-GACATGTATCGYGTGTGYATGGATAAGG-3’

L2 LGH8425 5- CCCGTGTTAGTGGTGACAGTCGTAAC-3’

L1 LGH8426 5’-CCGCCAACCAGGATGTAAGAAGTTG-3’

First round R1-L1 = 900-bp, second round R1-L2 = 820-bp, wsecond round B R2-L1 = 630-bp, third round AB R2-L2 = 450-bp.

EEHV1,2,5,6 Common RHS Extended U38(POL-C term) Locus:

R1 LGH7828 5’-CGACGGACARCARAGCAGRAT-3’ [Common EEHV1,2,5,6]

R2 LGH7831 5’-GCATTATACAGGCATACAACCTGTG-3’ [EEHV6-specific]

R3 LGH7829 5’-GGACATTCTTAACGCAAATAGCTCC-3’ [EEHV1,6 specific]

L5 LGH7830 5’-GGAACTTTCTAGTTGACATATCGCC-3’ [EEHV5 specific]

L4 LGH7483 5’-CTACATCTATACAGAACTTTCC-3’ [EEHV5-specific]

L3 LGH7584 5’-CATCGATTTTGAACTTCTCATGGTC-3’ [EEHV6-specific]

L2 LGH7833 5’-CCCACGTANCGYTTYTTRCA-3’ [EEHV1, 6]

L1 LGH7832 5’-CAAYTCTATNCCYTTCATNCT-3’ [EEHV2]

First round R1-L1 and R1-L1’ = 640-bp, multiple alternative internal first, second and third round options.

### EEHV3,4 U38(POL) Two-Step LHS and RHS Codehops Extended 1250-bp Locus:

| | | |
|---|---|---|
| R0 | LGH7926 | 5‘-GG[C/G]TACAACATCAACGC[C/G]TTCGA-3‘ |
| R1 | LGH7400 | 5‘-CAGCATCATCCAGGCGTACAAC-3‘ |
| L0 | LGH7928 | 5‘-CGAGGGTGGAGTAGCAGAGGTTG-3‘ |
| R2 | LGH6720 | 5‘-ATCCTGGCGCAGCTGCTGAC-3‘ |
| L2 | LGH7907 | 5‘-CGAAGACCTTGTCYACCTCCAGC-3‘ |
| L1 | LGH7906 | 5‘-GCTTCACGAAGACCTTGTCYACC-3‘ |

First round RHS extended codehops R1/L2 = 600-bp; second round A R1/L2 = 540-bp; second round B R2/L1 = 480-bp; third round R2/L2 = 440-bp. LHS extended first round R0/L0 = 630-bp.

### EEHV1,2,5,6 Specific and Common U48.5(TK) Locus:

| | | | |
|---|---|---|---|
| R1 | LGH7981 | 5’-CTRCATT[G/T][A/C]CCAAAGTATGGAAGTA-3’ | [EEHV1,2,6] |
| R2 | LGH7982 | 5’-CRTYTATATCATCAAARACYTCACA-3’ | [EEHV1B,2,6] |
| R3 | LGH9255 | 5’-CATCTAATTCATCAAACACCTCACAAGG-3’ | [EEHV2,5B] |
| R4 | LGH7983 | 5’-GCAGTGGTTT[G/T]ATCCARATAYGGTG-3’ | [EEHV1,2,6] |
| R5 | LGH8417 | 5’-CATCTAATTCATCAAACACCTCACAAGG-3’ | [EEHV2,5] |
| R6 | LGH9254 | 5‘-CCAAAATATGGTAGAATTTTCTCATC-3‘ | [EEHV2 only] |
| L10 | LGH8413 | 5‘-CGTCAACCAGAATAACGTCATGGGTGC-3‘ | [EEHV6] |
| R7 | LGH8416 | 5‘-CTGGTGTTCTAAAACAAGATTCGCTGTG-3‘ | [EEHV2,5] |
| L9 | LGH7984 | 5‘-CAGCCTTCAAGCGGCATACACTG-3‘ | [EEHV6] |
| L8 | LGH7985 | 5‘-GGTAGGTTCACCTACATGGAACTTC-3‘ | [EEHV6] |
| R8 | LGH6764 | 5‘-GCACG[A/G]TACCACGTACTC-3‘ | [Common] |
| R9 | LGH7970 | 5‘-TGC[A/C]GC[C/T]TGAAGGCTGTTTATATACT-3‘ | [Common] |
| L7 | LGH9253 | 5‘-AAACAGCCTYCARGC[Y/G]GCATATACTG-3‘ | [Common] |
| L6 | LGH8415 | 5‘-GAGTACGTGGTATCGTGCCAAAGAGTACC-3‘ | [EEHV2,5] |

| | | | |
|---|---|---|---|
| R10 | LGH4944B | 5‘-CAGCGTTTCCTCAGAACATCCCTGTAA-3‘ | [Common] |
| L5 | LGH9252 | 5‘-CAGGG[A/T]TGTTC[C/G]GAAGAAACGCTGARAGAG-3‘ | [EEHV1,2] |
| L4 | LGH8414 | 5‘-GATGTTATCTC[A/C]ATAGCCGATAGGCATG-3‘ | [EEHV2,5] |
| L3 | LGH7969 | 5‘-GCTGCGACAAAGTTGACAGGCATAATGG-3‘ | [EEHV2,5] |
| L2 | LGH7968 | 5‘-GCGGCAACGAAGTTCACAGGCATYATG-3‘ | [Common] |
| L1 | LGH4962 | 5‘-CAGATTAGCCTCGAAATATGTAGCGAA-3‘ | [Common] |

Appropriate combinations of primers were selected based on the order listed from right to left across this locus encompassing the complete U48.5(TK) ORF from EEHV1(Kimba) coordinates 105,835 to 106,908.

### EEHV5A plus EEHV5B Specific U51(vGPCR1) Gene Locus.

| | | |
|---|---|---|
| R1 | LGH9351 | 5’-GCGTTTAACGC[A/C]AC[A/G]CAGTTGGT-3’ |
| R2 | LGH9324 | 5’-CGCCACACAGTTGGTCGAAGCGTATGTG-3’ |
| R3 | LGH9367 | 5’-CCTCTATGGGTGTATCAGGCA-3’ |
| L2 | LGH9368 | 5’-CTCTCATA[C/T]TCCAC[A/G]TG[A/C]ACACAACA-3’ |
| L1 | LGH9352 | 5’-GTATTCGTAATTGGGATTCTGTTCTCC -3’ |

First round PCR R1-L1 = 990-bp; second round 2A R1-L2 = 930-bp; second round 2B R2-L1 = 980-bp; third round 3A R3-L1 = 810-bp; third round 3B R2-L2 = 920-bp; third round 3C R3-L2 = 750-bp.

### EEHV1,2,5,6 U70(EXO)/U71/U72(gM) Gene Locus Primers:

| | | | |
|---|---|---|---|
| R1 | LGH6749 | 5’-CTATGGGATCCGAACTTTC-3’ | [common] |
| R2 | LGH6750 | 5’-CTTTCTAAGGGGGTTTGTTGC-3’ | [common] |
| L2 | LGH6752 | 5’-CTACATGCCCATGCAGATAGG-3’ | [common] |
| L1 | LGH6751 | 5’-GAAGTCCTGCTAGCCCC[C/T]TAC-3’ | [EEHV1,5 only] |

First round PCR R1/L1 = 750-bp; second round R1/L2 = 730-bp; alternate second round R2/L1 = 730-bp; third round R2/L2 = 710-bp. Omit L1 for EEHV2 and EEHV6.

EEHV3,4 Specific U71(MyrTeg)-U72(gM) Gene Overlap Locus:

R1 LGH6793B 5’-GCCATCGGGATCCGGAAAA[A/G]ACGCC-3’

R2 LGH9263B 5’-GGACTGAAGGACAAAGACGG[A/G]CAGCC-3’

L2 LGH9210 5’-CCAGTACGATAAGATCTACCTGGACGA-3’

L1 LGH6792B 5’-GGGCATCTTCTTCACCCTCATAGCCATC-3’

First round PCR R1-L1 = 770-bp; second round 2A R1-L2 = 730-bp; second round 2B R2-lL1 = 680-bp; third round PCR R2-L2 = 640-bp

EEHV1,2,5,6 U73(OBP) Central Segment Locus:

Consensus EEHV1A,1B,2,5.6 non-redundant primers:

R1 LGH6572 5’-CGAGCTCCTATGGGATCTGG-3’

R2 LGH6573 5’-CAGATAGAAAGTTTATATAGAC-3’

L2 LGH6575 5’-CAAAACTGATGCCGACAGT-3’

L1 LGH6574 5’-GACATCATGTCGGGACC-3’

Added EEHV3,4 consensus primer:

L3 LGH6736 5’-CCGACGTACGAGTT[T/G]AGG-3’

First round PCR R1-L1 = 825-bp; 2cd round R1-L2 = 755-bp, second round R2-L1 = 615-bp, third round A R1-L2 = 545-bp, alternative third round B R1-L3 = 470-bp.

EEHV3-Specific U73(OBP) Primers:

R1 LGH6733 5’-CAGATCGAGAGCTTATATAGAC-3’

L1 LGH6718 5’-GTCCGGGAACCGGTTCCTGATC-3’

L2 LGH6736 5’-CCGACGTACGAGTT(G/T)AGG-3’

First round PCR R1-L1 = 500-bp; second round R1-L2 = 260-bp.

EEHV1A,1B,2,5,6 U77(HEL) Central Segment Locus:

R1 LGH6729 5’-CCGCRAGGATGGTCGTCTTC-3’

R2 LGH6743 5’-GCAAGGTRGAACGTATCG-3’

R3 LGH6741 5’-GTCCGTTCACCGCGCT[A/C]GC-3’

L2 LGH6744 5’-CCATAGA[T/G]ACGTGTCGAG-3’

L1 LGH6742 5’-CACAG[A/C]GCGTTGTAGAACC-3’

Added EEHV3 consensus primer:

R4 LGH6789 5’-CGATCCACAAGGAGTTCGG-3’

First round PCR R1-L1 = 800-bp, second round A R2-L1 = 580-bp, third round A R2-L2 = 350-bp; second or third round B R3-L1 = 440-bp, alternate third round R4-L1 = 290-bp.

EEHV3,4-Specific U76(POR)-U77(HEL)_ Primers:

R1 LGH7898 5’-GGGATGTTCGACACGGGGTTC-3’

R2 LGH7899 5’-CATGTGTTCGGCCAGCAAGGTCG-3’

L2 LGH7902 5’-GCGTGCAGCATGTGCTCCAGGATG-3’

L1 LGH7903 5’-GTACATGAACACGGCGTGTGCAG-3’

First round PCR R1-L1 = 570-bp; second round R1-L2 = 540-bp; alternate second round R2-L1 = 550-bp; third round R2-L2 = 500-bp.

EEHV2,5,6 Specific and Consensus Overlapping U81(UDG)-U82(gL) Locus:

Initially designed based on EEHV2 data only but later ones on commonalities between EEHV1A and EEHV2. Individual primers below are known to be effective for the following EEHV species given in parentheses.

R1 LGH9360 5’-GACGAYCTGATTAAAGAACACRCGGA-3’ [1A, 2, 5B]

|  |  |  |  |
|---|---|---|---|
| R2 | LGH9462 | 5’-GACCA[A/C]TTATAGGYGCYGTRAAACC-3’ | [1A, 6 only] |
| R3 | LGH9335 | 5’-CCAYTAGTGTCGTTGTRRGRTGTGAT-3’ | [1A, 2] |
| L7 | LGH9361 | 5’-CGATCACAYCYYACAACGACACTARTGG-3’ | [1A, 2, 5B] |
| R4 | LGH9336 | 5’-CTCTGAATGATAAATGGAAYCTG-3’ | [1A, 2] |
| R5 | LGH9362 | 5’-GTGGTTATTTCC[A/T]ACRAAACCTGCG-3’ | [Common] |
| L6 | LGH9464 | 5’-CACCTTTAGCCGCGACCAGGTACGCAGG-3’ | [1A, 6, 2] |
| R6 | LGH9363 | 5’-GGYTGRGGATACGGRTCCTGTCCAAC-3’ | [1A, 2] |
| R7 | LGH9617 | 5’-GGCAAAGGTAGCTCCATCTATGTAC-3’ | [2, 5A] |
| R8 | LGH9389 | 5’-TTGGGAGCAGGGTATATTATCTCCAG-3’ | [1A, 2, 5B, 6] |
| L5 | LGH9364 | 5’-GTRGAGGAGCAG[A/C]GRTC[A/T]CTGGAGA-3’ | [Common] |
| R9 | LGH9337 | 5’-GGGCGTGGCTAG[A/T][A/T]GGRTCRCGATACAGC-3’ | [Common] |
| L4 | LGH9338B | 5’-GCTGTATCGYGAYCC[A/T][A/T]CTAGCCACGCCC-3’ | [2, 5B, 6] |
| L3 | LGH9619 | 5’-GCATTCCGCTACGGACACGACTC-3’ | [5A only] |
| L2’ | LGH9339 | 5’-CGACATGTTACATCATGGGGTGTATGT-3’ | [2, 5B only] |
| L2 | LGH9341 | 5’-AGACACGCTACGTCTTGGGGAGTGTGT-3’ | [1A, 6 only] |
| L1’ | LGH9340 | 5’-CACAATACGTGGTACAAATATCCACA-3’ | [2, 5A only] |
| L1 | LGH9342 | 5’-CTCCAAACTTGGTACAAATATCCACA-3’ | [1A, 6 only] |

Appropriate combinations of primers were chosen based on the positions listed in order from right to left across this overall 2.9-kb locus corresponding to EEHV1(Kimba) genomic coordinates 144,912 to 147969.

## Supplemental Material: Section 3: Full or Alternative Virus Names and Non-EEHV Genbank Accession Numbers:

Explanations of abbreviated virus names and Genbank accession numbers for selected non-EEHV reference genes used in the phylogenetic tree dendrograms in **Figures 6, 7 and 8** were as follows: African green monkey cytomegalovirus (AGMCMV) FJ483968; baboon cytomegalovirus or papiine HV# (BabCMV) AC090446;

baboon lymphocryptovirus or papiine HV# (BabLCV2) AF534229; cebus monkey CMV or cebine HV1 (CMCMV) JQ264772; chimpanzee alphaherpes simplex virus or panine HV# (ChHSV) NC_023677; chimpanzee cytomegalovirus or panine HV2 (ChCMV, Haberling) AF480884; chimpanzee (pan) lymphocryptovirus (PpanLCV1) AF534220; equine alphaherpesvirus 1 (EHV1) AY665713; equine alphaherpesvirus 4 (EHV4) AF030027; gorilla cytomegalovirus 2.2 (GorCMV) FJ538490; gorilla lymphocryptovirus 1 (GorLCV1) AF534225; gorilla lymphocryptovirus 2 (GorLCV2) AY129395; guinea-pig cytomegalovirus or caviid HV2 (GPCMV) KC503762; human cytomegalovirus (HCMV) AY446894; human lymphocryptovirus or Epstein Barr virus (EBV) AJ507799; human roseolovirus 6A (HHV6A) X83413; human roseolovirus 6B (HHV6B) AF157706; human roseolovirus 7 (HHV7) AF037218; human alphaherpes simplex virus 1 (HSV1) X14112; human alphaherpes simplex virus 2 (HSV2) Z86099; infectious bovine rhinotracheitis alphaherpesvirus (IBRV) or bovine HV1 AJ004801; human Kaposi's sarcoma rhadinovirus (KSHV or HHV8) U75698; Marek's disease alphaherpesvirus or Gallid HV2 (MDV) AF243438; marmoset alphaherpesvirus or saimirine HV1 (MarHV1) HM625781; marmoset lymphocryptovirus or callitrichine HV3 (CalHV3) NC_004367; mouse cytomegalovirus or MuHV1 (MCMV) U68229; orang-utang lymphocryptovirus or pongine HV# (PpygLCV1), owl monkey cytomegalovirus or aotine HV# (OMCMV) FJ483970; porcine pseudorabies alphaherpesvirus (PRV) BK001744; rat cytomegalovirus or MuHV2 (RCMV) AF2328689; rhesus (macaquine) alphaherpesvirus 1 or B-virus (RhBV1) AF533768; rhesus cytomegalovirus (RhCMV) AY186194; rhesus lymphocryptovirus (RhEBV, RhLCV2) AY037858; squirrel monkey cytomegalovirus or saimiriine HV# (SQCMV) FJ483967; squirrel monkey rhadinovirus, HV*saimiri* or saimiriine HV2 (HVS) X64346; tree shrew cytomegalovirus or HVt*upaia* or tupaiid HV1/2? (HVTup) AF281817; green turtle alphaherpesvirus (TurtleHV) AF035003; human varicella zoster alphaherpesvirus (VZV) X04370.

## Supplemental Material: Section 4: EEHV Genbank Accession Numbers for EEHV DNA or Protein Files Used in the Phylogenetic Dendrograms or Clustal Comparisons:

**Figure 2:** For DNA file accession numbers used here see **Table S1**.

**Figure 6 (DNA): Panel (a) U38(POL)** locus, EEHV1A(#NAP11) HM568510, EEHV1A(#NAP18) HM568523, EEHV1B(#NAP14) HM568536, EEHV1B(#NAP19) HM568550, EEHV2(#NAP12) HM568558, EEHV3(#NAP27) JN983092, EEHV4(#NAP22) JN983096, EEHV5A(#NAP28) JN983100, EEHV5A(#NAP50) JN983108, EEHV5B(#NAP58) JX011013, EEHV6(#NAP35) JF692762 ; **(b) U73(OBP)** locus, EEHV1A(#NAP11) HM568515, EEHV1A(#NAP18) HM568528, EEHV1B(#NAP14) HM568541, EEHV1B(#NAP19) HM568556, EEHV2(#NAP12) HM568564, EEHV3(#NAP27) JN983094, EEHV4(#NAP22), EEHV5A(#NAP28) JN983098, EEHV5A(#NAP50) JN983114, EEHV5B(#NAP58) KC854723, EEHV6(#NAP35) JN983124; **(c) U76(POR)-U77(HEL)** locus, EEHV1A(#NAP11) HM568515, EEHV1A(#NAP18) HM568529, EEHV1B(#NAP14) HM568542, EEHV1B(#NAP19) HM568557, EEHV2(#NAP12) HM568564, EEHV3(#NAP27) JN983095, EEHV4(#NAP22) JN983099, EEHV5A(#NAP50) JN983115, EEHV5B(#NAP58) JX011029, EEHV6(#NAP35) JN983126; and **(d) U71-U72(gM)** locus, EEHV1A(#NAP11) HM568515, EEHV1A(#NAP18) HM568528, EEHV1B(#NAP14) HM568541, EEHV1B(#NAP19) HM568556, EEHV2(#NAP12) HM568564, EEHV3(#NAP27) JN983093, EEHV4(#NAP22) JN983097, EEHV5A(#NAP28) JN983105, EEHV5A(#NAP50) JN983113, EEHV5B(#NAP58) JX011024, EEHV6(#NAP35) JN983124.

**Figure 7 (Protein): Panel: (a) U48.5(TK)**, thymidine kinase, EEHV1A(#NAP11) ADK70792, EEHV1A(#NAP18) ADK70833, EEHV1B(#NAP14) ADK70865, EEHV1B(#NAP19) ADK70900, EEHV2(#NAP12) ADK70928, EEHV5A(#NAP50) AEW50127, EEHV5B(#NAP58) AGZ171188, EEHV6(#NAP35) AEW50144; **(b) U27(PPF)**, EEHV1A(#NAP11) ADK70789, EEHV1B(#NAP14) ADK70869, EEHV2(#NAP12) ADK70923, EEHV5A(#NAP50) AGK82349, EEHV5B(#NAP58) AGZ17190, EEHV6(#NAP35) AEW50139; **(c) U39(gB)**, EEHV1A(#NAP11) ADK70785, EEHV1A(#NAP18) ADK70824, EEHV1B(#NAP14) ADK70861, EEHV1B(#NAP19) ADK70891, EEHV2(#NAP12) ADK70917, EEHV5A(#NAP50) AGK82348, EEHV5B(#NAP58) AFO11059, EEHV6(#NAP35) AEM72556; **(d) U82(gL)**, EEHV1A(#NAP18) AGZ17099, EEHV1A(#NAP11) AGZ17104, EEHV1B(#NAP14) AIC84322, EEHV1B(#NAP19) AIC84232, EEHV2(#NAP12) AGZ17124, EEHV5A(#NAP50) AGZ17132, EEHV5B(#NAP58) AGZ17140, EEHV6(#NAP35) AGZ17150; and **(e) U81(UDG)**, EEHV1A(#NAP18) AGZ17098, EEHV1A(#NAP11) AGZ17105, EEHV1B(#NAP14), EEHV1B(#NAP19), EEHV2(#NAP12) AGZ17122, EEHV5A(NAP50) AGZ17131, EEHV5B(#NAP58) AGZ17139, EEHV6(#NAP35), AGZ17150.

**Figure 8 (Proteins):** **U38 (POL):** EEHV1A(NAP18) ADK70825; EEHV1B(NAP14) ADK70862; EEHV2(NAP12) ADK70918; EEHV3(NAP27) AEW50096; EEHV4(NAP22) AEW50102; EEHV5A(NAP50) AEW50122; EEHV5B(NAP58) AGK82352; EEHV6(NAP35) AEM72555

**Figure 9 (Proteins):** **Panel (a): U82(gL),** EEHV1A(#NAP11) AGZ17104, EEHV1A(#NAP18) AGZ17099, EEHV1B(#NAP14) AIC84223, EEHV1B(#NAP19) AIC84232, EEHV6(#NAP35) AGZ17149, EEHV2(#NAP12) AGL61575, EEHV5A(#NAP50) AGZ17132 and EEHV5B(#NAP58) AGZ17140. **Panel (b): U81(UDG),** EEHV1A(#NAP11) AGZ17105, EEHV1A(#NAP18) AGZ17098, EEHV1B(#NAP14) AIC84222, EEHV1B(NAP19) AIC84231, EEHV6(#NAP35) AGZ17150, EEHV2(#NAP12) AGL61574, EEHV5A(#NAP50) AGZ17131 and EEHV5B(#NAP58) AGZ17139.

**Figure 10 (Proteins):** **Panel (a): U45.7(ORF-J)**, EEHV1A(#NAP11) ADK70790, EEHV1A(#NAP18) ADK70829, EEHV1B(#NAP14) ADK70870, EEHV1B(NAP19) ADK70896, EEHV6(#NAP35) AEW50140 and EEHV2(#NAP12) ADK70924. **Panel (b): U46(gN),** EEHV1A(#NAP11) ADK70791, EEHV1A(#NAP18) ADK70830, EEHV1B(#NAP14) ADK70871, EEHV1B(NAP19) ADK70897, EEHV6(#NAP35) AEW50141 and EEHV2(#NAP12) ADK70925. **Panel (c): U80.5(vCXCL1, ORF-N),**

EEHV1A(#NAP11) AGZ17107, EEHV1A(#NAP18) AGZ17101, EEHV6(#NAP35) AIC91262, EEHV2(#NAP12) AGZ17121, EEHV5A(#NAP50) AID07327 and EEHV5B(#NAP58) AGZ17138.

**Table S1:** Note that six sequenced PCR loci that are included represent expanded versions of smaller segment files reported earlier elsewhere under Genbank accession numbers EU658935(JN(983092) for EEHV3 POL, EU658934 (JN983096) for EEHV4 POL (3), as HM060764(JN983100) for EEHV5A(NAP28) POL and HM060765(JF692762) for EEHV6(NAP35) POL (4) and as JN983108 and JX983113 for EEHV5A(NAP50) and EEHV5B(NAP58) for U38/POL (17). For completeness, four previous unaltered loci EU658935 for EEHV3 TER and EU658935 for EEHV4 TER (3) as well as JX011013 for EEHV5A(NAP50) U71-gM and and JX011024 for EEHV5B(NAP58) U71-gM (17) are also included.