MATERIALS AND METHODS

Southern blot. HSV-BAC mini preps were performed from 10 ml overnight bacterial cultures by alkaline lysis followed by phenol:chloroform extraction. DNAs were digested with the appropriate restriction enzyme overnight, separated on 0.5% agarose gels, and transferred to positively charged nylon membranes (Roche). The blots were hybridized with the appropriate digoxigenin-dUTP-labeled DNA probe synthesized with the PCR DIG Labeling Mix (Roche), and detected with anti-digoxigenin-alkaline phosphatase followed by CDP-Star (disodium 4-chloro-3-(methoxyspiro {1,2-dioxetane-3,2-(5-chloro)tricyclo [3.3.1.1^{3,7}]decan}- 4-yl)phenyl phosphate) chemiluminescent substrate (Roche).

Determination of genome copy number by quantitative real-time PCR.

To quantitate the amount of viral genomes present in the viral stocks, and relative to the viral titer, a quantitative real-time PCR was performed on serial dilutions of crude viral stocks prepared from infected cell lysates, with primers designed on glycoprotein G coding sequence gG_504_f (CTT GGT TCC GAC GCC TCA ACA TAC) and gG_603_r (TAA GGT GTG GAT GAC GGT GCT GAC), using Bio-Rad iQ SYBR Green Supermix in a Bio-Rad iCycler thermocycler with the following protocol: 95 °C for 1' 30" (1 cycle), 95°C for 15" and 60 °C for 30" (50 cycles), followed by melt curve analysis (55 °C for 10", increase of setpoint temperature after cycle 2 by 0.5 °C, 80 cycles). gDminus-EGFP-HSV-BAC DNA was used as positive control and to generate the standard curve with 10-fold serial dilutions (from 10^8 to10^2 copies). To exclude inhibition of amplification by contaminants present in the crude lysates, 10^6 copies of the gDminus-EGFP-HSV-BAC DNA standard were spiked in replicate wells. The data were analyzed with iCycler software.

1 The figures in Table S1 indicate that there is a ratio of genome copies *vs* plaque forming units ranging from ~$3 \cdot 10^3$ to ~10^4. In order to provide cells with the same amount of input virus, all the experiments were performed with equivalent m.o.i. for each virus, as measured by titration in SKOV3 cells.

Table S1.

Recombinant	Cell line used to prepare viral stock	Genome copies / µl of crude viral stock mean ± S.D.	Viral stock titer (pfu / ml)	Genome copies x 10^3 / pfu
R-LM5	BHK	$1.82 \pm 0.17 \cdot 10^9$	$6.3 \cdot 10^8$	2.9
R-LM13	BHK	$1.28 \pm 0.39 \cdot 10^9$	$1.2 \cdot 10^8$	10.7
R-LM39	J-HER2	$2.18 \pm 0.05 \cdot 10^8$	$5.1 \cdot 10^7$	4.3
R-LM113	J-HER2	$4.00 \pm 0.28 \cdot 10^9$	$5.3 \cdot 10^8$	7.5

FIGURE LEGEND

Fig. S1. (A) Agarose gel electrophoresis of molecular weight markers (MW), and the following BamHI-digested DNAs: shuttle plasmid pS31 (lane 1), HSV-1 BAC plasmids pYEbac102 (lane 2), 102 gD^{-}FRT (lane 3), gDminus-LacZ-HSV-BAC (lane 4), the cointegrate resulting from insertion of pS31 into gDminus-LacZ-HSV-BAC (lane 5), and the resolved cointegrate LM31-BAC (lane 6).

(B) Autoradiographic image of the digests of panel A hybridized to a DIG-labeled probe designed on the scHER2 sequences, PCR amplified from plasmid pS31. In lane 1 the probe hybridizes to one band of the digested plasmid (7771 bp). A fragment of the same size is generated upon digestion of the cointegrate BAC (lane 5), whereas digestion of the resolved recombinant LM31-BAC generates a 3059 bp band (lane 6). The position of the primers used to synthesize the probe is shown in the box.

(C) Schematic diagram of the procedure employed to generate recombinant BACs carrying gD-scHER2L. (a, b) Diagram of the structure of pYEbac102 genome, showing the position of the insertion of BAC sequences (line a) into the genome (line b) (S1). (c) Detail of the BamHI J fragment (Bam J), containing gD (grey box). gD upstream and downstream flanking sequences are shown as hatched and dotted boxes, respectively. (d) Structure of the Bam J fragment following gD gene deletion by FLP recombinase-mediated excision. (e, f) pS31 shuttle plasmid used for homologous recombination in E.coli: scHER2L is represented as a black box. (g) Structure of modified BamJ fragment in the cointegrate resulting from insertion of pS31 shuttle vector into gDminus-LacZ-HSV-BAC *via* gD downstream flanking sequences: the insertion introduces two additional BamHI sites. (h) Structure of the modified BamJ fragment in the resolved cointegrate resulting from excision of pS31 shuttle vector v*ia* gD upstream flanking sequences: the excision removes one of the two BamHI sites added in the cointegrate. B, BamHI restriction sites. Figures below the arrows indicate the expected fragment lengths in bp. Boxed figures refer to sizes of the fragments that hybridize to the probe in panel B.

REFERENCE

S1. **Tanaka, M., H. Kagawa, Y. Yamanashi, T. Sata, and Y. Kawaguchi.** 2003. Construction of an excisable bacterial artificial chromosome containing a full-length infectious clone of herpes simplex virus type 1: viruses reconstituted from the clone exhibit wild-type properties in vitro and in vivo. J Virol **77:**1382-91.